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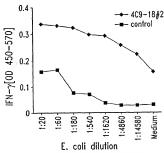
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[Continued on next page]

(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION



(57) Abstract: Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided. together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

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Without international search report and to be republished upon receipt of that report.

# COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

### TECHNICAL FIELD

The present invention relates generally to the detection and treatment of

Chlamydial infection. In particular, the invention is related to polypeptides comprising

a Chlamydia antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

# BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. Chlamydia trachomatis is one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. Chlamydia trachomatis may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with 15 Chlamydia trachomatis, is the leading cause of preventable blindness worldwide. Chlamydia pneumonia is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to Chlamydia pneumonia have been shown to be at least twice as likely to suffer from 20 coronary heart disease as seronegative individuals. Chlamydial infections thus constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and parmaceutical compositions for the prevention and treatment of Chlamydia infections. The present invention fulfills this need and further provides other related advantages.

### SUMMARY OF THE INVENTION

The present invention provides compositions and methods for the diagnosis and therapy of Chlamydia infection. In one aspect, the present invention 30 provides polypeptides comprising an immunogenic portion of a Chlamydia antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises an amino acid

sequence encoded by a polynucleotide sequence selected from the group consisting of
(a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122,
124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the
complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or
(b) under moderately stringent conditions. In specific embodiments, the polypeptides
of the present invention comprise at least a portion of a *Chlamydial* protein that
includes an amino acid sequence selected from the group consisting of sequences
recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167,
168, 224-262, 246, 247, 254-256, 292, 294-305 and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a *Chlamydial* protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above
15 polypeptides, recombinant expression vectors comprising one or more of these
polynucleotide sequences and host cells transformed or transfected with such expression
vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a 20 known Chlamydia antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a Chlamydlal protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more Chlamydia polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines for prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines. WO 01/40474 PCT/US00/32919

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In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polyneptide) to 5 provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting 10 cells to the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, B-cells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, within other aspects, methods for removing Chlamydial-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a Chlamydial protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of Chlamydial infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting Chlamydia infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting Chlamydia infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

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The present invention also provides methods for detecting Chlamvdia infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence 5 disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting Chlamydia infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. 15 embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated 20 individually.

# SEQUENCE IDENTIFIERS

10

30

SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone 1-B1-66.

SEO ID NO: 2 is the determined DNA sequence for the C. trachomatis 25 clone 4-D7-28.

SEO ID NO: 3 is the determined DNA sequence for the C. trachomatis clone 3-G3-10.

SEO ID NO: 4 is the determined DNA sequence for the C. trachomatis clone 10-C10-31.

> SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66. SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28. SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10. SEO ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEO ID NO: 9 is a third predicted amino acid sequence for 3-G3-10. 35

5 B1-66/48-67.

10

SEQ ID NO: 10 is a fourth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-

SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-

B1-66/58-77. SEQ ID NO: 15 is the determined DNA sequence for the *C. trachomatis* 

SEQ ID NO: 15 is the determined DNA sequence for the C. Irachomans servar LGV II clone 2C7-8

SEQ ID NO: 16 is a DNA sequence of a putative open reading frame from a region of the C. trachomatis servor D genome to which 2C7-8 maps

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the DNA sequence of SEQ ID NO: 16

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide

15 CtC7.8-12

SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from C. trachomatis serovar D

20 SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from
C. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from *C. trachomatis* LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from C. trachomatts LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from *C. trachomatis* LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from *C. trachomatis* LGV II

30 SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from C. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from 35 C. pneumonta strain TWAR

SEQ ID NO: 29 is the determined DNA sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 30 is the predicted amino acid sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 31 is the amino acid sequence for a 10mer consensus peptide from CtC7.8-12 and CtC7.8-13

SEQ ID NO: 32 is the predicted amino acid sequence for clone 2C7-8 from C. trachomatis LGV II

SEQ ID NO: 33 is the DNA sequence corresponding to nucleotides

10 597304-597145 of the *C. trachomatis* serovar D genome (NCBI, BLASTN search),
which shows homology to clone 2C7-8

SEQ ID NO: 34 is the predicted amino acid sequence encoded by the sequence of SEQ ID NO: 33  $\,$ 

SEQ ID NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer)

15 from C. pneumonia SEQ ID NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from C. pneumonia

20 SEQ ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of *C. trachomatis* 

30 SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of C. pneumonia

SEQ ID NO: 44 is a first determined DNA sequence for the C. trachomatis LGV II clone 19783.3.jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the C

35 trachomatis LGV II clone 19783.4.jen.seq(1>481)CTL2#11-5', representing the 5' end.

SEQ ID NO: 46 is the determined DNA sequence for the *C. trachomatis* LGV II clone19784CTL2\_12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the C. trachomatis LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

5 SEQ ID NO: 48 is a first determined DNA sequence for the C. trachomatis LGV II clone 19786.3,jen.seq(1>600)CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the C trachomatis LGV II clone 19786.4,jen.seq(1>600)CTL2#18-5', representing the 5' cnd.

SEQ ID NO: 50 is the determined DNA sequence for the *C. trachomatis*10 LGV II clone 19788CTL2 21consensus.seq(1>406)CTL2#21.

O LGV II clone 19/88C1L2\_21consensus.seq(1>400)C1L2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis*LGV II clone 19790CTL2\_23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19791CTL2\_24consensus.seq(1>145)CTL2#24.

15 SEQ ID NO: 53 is the determined DNA sequence for the C. trachomatis LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis*20 LGV II clone15-G1-89, sharing homology to the lipoamide dehydrogenase gene
CT557.

SEQ ID NO: 56 is the determined DNA sequence for the *C. trachomatis* LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the *C. trachomatis*25 LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the C. trachomatis
30 LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

SEQ ID NO: 60 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H3-68, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the *C. trachomatis*35 LGV II clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene CT376 and to the glycogen hydrolase gene CT042.

SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

5 SEQ ID NO: 64 is the determined DNA sequence for the C. trachomatis LGV II clone 11-A3-93, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the *C. trachomatis* LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

10 SEQ ID NO: 66 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the  $\it C.$  trachomatis LGV II clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the C. trachomatis

15 LGV II clone CtL2#6. SEQ ID NO: 69 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#2.

20 SEQ ID NO: 71 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the C. trachomatis LGV II clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the C. trachomatis LGV II clone 23509.1CiL2#3-3', representing the 3' end.

SEQ ID NO: 74 is a first determined DNA sequence for the C. trachomatis LGV II clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the C. trachomatis LGV II clone 22121.1CtL2#10-3', representing the 3' end.

30 SEQ ID NO: 76 is the determined DNA sequence for the C. trachomatis LGV II clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the  $\it C.\ pneumoniae$  LGV II clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the *C. pneumoniae* 35 LGV II clone Cp SWIB-His.

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SEQ ID NO: 79 is the determined DNA sequence for the *C. trachomatis*LGV II clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the *C. trachomatis*5 LGV II clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the *C. trachomatis* 

LGV II clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the C. trachomatis

SEQ ID NO: 82 is the determined DNA sequence for the C. trachomatis. LGV II clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the  $\it C. trachomatis$  LGV II clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

SEQ ID NO: 84 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the C. trachomatis LGV II clone 17-E11-72, sharing partial homology to the OppC\_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the *C. trachomatis* LGV II clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

20 SEQ ID NO: 87 is the determined DNA sequence for the C. trachomatis LGV II clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the *C. trachomatis* LGV II clone 15-A3-26, sharing homology to the CT858 ORF.

25 SEQ ID NO: 89 is the determined amino acid sequence for the C. pnuemoniae clone Cp SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the C. trachomatis LGV II clone CtL2 LPDA FL.

SEQ ID NO: 91 is the determined amino acid sequence for the C. 30 pnuemoniae clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the  $\it C.trachomatis$  LGV II clone Ctl.2\_TSA\_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

35 SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from C. trachomatis LGV II.

SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from C. trachomatis LGV II.

5 SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from C. trachomatis LGV II.

SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.

SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide

10 from C. pneumonia. SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from C. pneumonia.

SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from C. pneumonia.

15 SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide from C. pneumonia.

SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.

SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide 20 from C. trachomatis.

SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.

SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from C. trachomatis.

25 SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from C. trachomatis.

SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide from C. pneumoniae.

SEQ 1D NO: 110 is the determined DNA sequence for the *C. trachomatis* LGV II clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.

SEQ ID NO: 111 is the determined DNA sequence for the C.

35 trachomatis LGV II clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

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SEQ ID NO: 112 is the determined DNA sequence for the C. trachomatis LGV II clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the C.

5 trachomatis LGV II clone 17-E2-9, sharing partial homology to the CT611 and CT 610

ORFs

SEQ ID NO: 114 is the determined DNA sequence for the C. trachomatis LGV II clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the C. 10 trachomatis LGV II clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the  $\it C$ .  $\it trachomatis$  LGV II clone 20-G3-45, containing part of the pmpB gene CT413.

is LGV II clone 20-G3-45, containing part of the pmpB gene C1415.

SEQ ID NO: 117 is the determined DNA sequence for the C.

trachomatis LGV II clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.
SEQ ID NO: 118 is the determined DNA sequence for the C.
trachomatis LGV II clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the *C. trachomatis* LGV II clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of 20 the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the  $\it C$ -trachomatis serovar LGV II Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the C. trachomatis serovar LGV II Cap1 gene CT529.

25 SEQ ID NO: 122 is the determined full-length DNA sequence for the C. trachomatis serovar E Cap1 gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the  $\it C. trachomatis$  serovar E Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the C. trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the C. trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the  $\it C$ .  $\it trachomatis$  serovar G Cap1 gene CT529.

35 SEQ ID NO: 127 is the predicted full-length amino acid sequence for the C. trachomatis serovar G Cap1 gene CT529. SEQ ID NO: 128 is the determined full-length DNA sequence for the C. trachomatis serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the C. trachomatis serovar F1 NII Cap1 gene CT529.

5 SEQ ID NO: 130 is the determined full-length DNA sequence for the C. trachomatis serovar L1 Cap1 gene CT529.

SEQ ID NO: 131 is the predicted full-length amino acid sequence for the C. trachomatis serovar L1 Cap1 gene CT529.

SEQ ID NO: 132 is the determined full-length DNA sequence for the C.

10 trachomatis serovar L3 Cap1 gene CT529.
SEO ID NO: 133 is the predicted full-length amino acid sequence for the

C. trachomatis serovar L3 Cap1 gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the  $\it C.$  trachomatis serovar Ba Cap1 gene CT529.

15 SEQ ID NO: 135 is the predicted full-length amino acid sequence for the C. trachomatis serovar Ba Cap1 gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the C. trachomatis serovar MOPN Cap1 gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the 20 C. trachomatis serovar MOPN Cap1 gene CT529.

SEQ 1D NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of C. trachomatis serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Cap1

CT529 ORF peptide #132-147 of *C. trachomatis* serovar L2.
25 SEQ ID NO: 140 is the determined amino acid sequence for the Cap1

CT529 ORF peptide #138-155 of *C. trachomatis* serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Capl

CT529 ORF peptide #146-163 of *C. trachomatis* serovar L2.

SEO ID NO: 142 is the determined amino acid sequence for the Cap1

30 CT529 ORF peptide #154-171 of C. trachomatis serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Capl CT529 ORF peptide #162-178 of C. trachomatis serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Capl CT529 ORF peptide #138-147 of *C. trachomatis* serovar L2.

35 SEQ ID NO: 145 is the determined amino acid sequence for the Capl CT529 ORF peptide #139-147 of C. trachomatis serovar L2. WO 01/40474

25 serovar L2.

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SEQ ID NO: 146 is the determined amino acid sequence for the Capl CT529 ORF peptide #140-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Capl CT529 ORF peptide #138-146 of C. trachomatis serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Capl CT529 ORF peptide #138-145 of *C. trachomatis* serovar L2.

SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF pentide # F140->I of C. trachomatis serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Capl

10 CT529 ORF peptide ##S139>Ga of C. trachomatis serovar L2.
SEO ID NO: 151 is the determined amino acid sequence for the Capl

CT529 ORF peptide ##S139>Gb of C. trachomatis serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of C. trachomatis serovar L2.

15 SEQ ID NO: 153 is the determined amino acid sequence for the peptide #2 C7.8-7 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide 20 #2 C7.8-9 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide #2 C7.8-10 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of C. trachomatis

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) 30 primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

35 SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

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SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer 10 epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the C. trachomatis pmpl gene.

15 SEQ ID NO: 170 is the determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the  $\it C$ .  $\it trachomatis pmpD$  gene.

SEQ ID NO: 173 is the determined full-length DNA sequence for the  $\it C.$  trachomatis pmpC gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

25 SEQ ID NO: 175 is the predicted full-length amino acid sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 176 is the predicted full-length amino acid sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the

30 C. trachomatis pmpE gene.
SEQ ID NO: 178 is the predicted full-length amino acid sequence for the

C. trachomatis pmpD gene.

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the *C. trachomatis* pmpC gene.

35 SEQ ID NO: 180 is the predicted full-length amino acid sequence for the C. trachomatis pmpB gene.

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SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpl gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the *C. trachomatis* pmpG gene.

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the 10 amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the C. trachomatis pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the C. trachomatis pmpC gene.

15 SEQ ID NO: 188 is the determined DNA sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the 20 C. trachomatis pmpG gene.

SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the  $\it C. trachomatis$  pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the C. truchomatis pmpD gene.

25 SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the C. pneumoniae serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.

35 SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

- SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpC gene in the SKB vaccine vector.
- SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.
- 5 SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.
  - SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpD gene in the SKB vaccine vector.
- SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo
- 10 primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector. SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.
  - SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.
- 15 SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.
  - SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.
- 20 SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.
- SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the 25 pET17b vector.
  - SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.
- SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo 30 primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.
  - SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo 10 primer for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

15 SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpl gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the *C. trachomatis* pmpl gene in the pET17b vector.

25 SEQ ID NO: 224 is the determined amino acid sequence for the C. pneumoniae Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the C. 30 pneumoniae Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 22-41.

35 SEQ ID NO: 229 is the determined amino acid sequence for the C. pneumoniae Swib peptide 27-46.

- SEQ ID NO: 230 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 42-61.
- SEQ ID NO: 231 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 46-65.
- 5 SEQ ID NO: 232 is the determined amino acid sequence for the C. pneumoniae Swib peptide 51-70.
  - SEQ ID NO: 233 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 56-75.
- SEQ ID NO: 234 is the determined amino acid sequence for the C.

  pneumoniae Swib peptide 61-80.
- SEQ ID NO: 235 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 66-87.
  - SEQ ID NO: 236 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 103-122.
- 15 SEQ ID NO: 237 is the determined amino acid sequence for the C. trachomatis OMCB pertide 108-127.
  - SEQ ID NO: 238 is the determined amino acid sequence for the  $\it C$ . trachomatis OMCB peptide 113-132.
- SEQ ID NO: 239 is the determined amino acid sequence for the C. 20 trachomatis OMCB peptide 118-137.
  - SEQ ID NO: 240 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 123-143.
  - SEQ ID NO: 241 is the determined amino acid sequence for the C. trachomatis OMCB peptide 128-147.
- 25 SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.
  - SEQ ID NO: 243 is the determined amino acid sequence for the  $\it C$ . trachomatis OMCB peptide 137-156.
- SEQ ID NO: 244 is the determined amino acid sequence for the C. 30 trachomatis OMCB pertide 142-161.
  - SEQ ID NO: 245 is the determined amino acid sequence for the C. trachomatis OMCB peptide 147-166.
  - SEQ ID NO: 246 is the determined amino acid sequence for the *C. trachomatis* OMCB pcptide 152-171.
- 35 SEQ ID NO: 247 is the determined amino acid sequence for the C. trachomatis OMCB peptide 157-176.

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SEQ ID NO: 248 is the determined amino acid sequence for the C. trachomatis OMCB peptide 162-181.

SEO ID NO: 249 is the determined amino acid sequence for the C. trachomatis OMCB peptide 167-186.

5 SEO ID NO: 250 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-190.

SEO ID NO: 251 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-186.

SEO ID NO: 252 is the determined amino acid sequence for the C.

10 trachomatis OMCB peptide 175-186. SEO ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.

SEO ID NO: 253 is the determined amino acid sequence for the C.

pneumoniae OMCB peptide 185-198. SEO ID NO: 254 is the determined amino acid sequence for the C. 15 trachomatis TSA peptide 96-115.

SEO ID NO: 255 is the determined amino acid sequence for the C. trachomatis TSA peptide 101-120.

SEQ ID NO: 256 is the determined amino acid sequence for the C.

trachomatis TSA peptide 106-125. 20 SEO ID NO: 257 is the determined amino acid sequence for the C.

trachomatis TSA peptide 111-130.

SEQ ID NO: 258 is the determined amino acid sequence for the C. trachomatis TSA peptide 116-135.

SEO ID NO: 259 is the determined amino acid sequence for the C. 25 trachomatis TSA peptide 121-140.

SEQ ID NO: 260 is the determined amino acid sequence for the C. trachomatis TSA peptide 126-145.

SEQ ID NO: 261 is the determined amino acid sequence for the C. 30 trachomatis TSA peptide 131-150.

SEO ID NO: 262 is the determined amino acid sequence for the C. trachomatis TSA peptide 136-155.

SEO ID NO: 263 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

35 SEO ID NO: 264 is the predicted full-length amino sequence for the C. trachomatis CT529/Cap 1 gene serovar I.

- SEQ ID NO: 265 is the determined full-length DNA sequence for the *C. trachomatis* CT529/Cap 1 gene serovar K.
- SEQ ID NO: 266 is the predicted full-length amino sequence for the *C. trachomatis* CT529/Cap 1 gene serovar K.
- SEQ ID NO: 267 is the determined DNA sequence for the C. trachomatis clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.
- SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the *C. trachomatis* CT016 gene in clone 2E10.
- SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the C. trachomatis tRNA syntase gene in clone 2E10.
  - SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the C. trachomatis clpX gene in clone 2E10.
- SEQ ID NO: 271 is a first determined DNA sequence for the *C.* 15 trachomatis clone CtL2gam-30 representing the 5'end.
  - SEQ ID NO: 272 is a second determined DNA sequence for the *C. trachomatis* clone CtL2gam-30 representing the 3'end.
  - SEQ ID NO: 273 is the determined DNA sequence for the C. trachomatis clone Ctl.2gam-28.
- 20 SEQ ID NO: 274 is the determined DNA sequence for the C. trachomatis clone Ctl.2gam-27.
  - SEQ ID NO: 275 is the determined DNA sequence for the  $\it C$ .  $\it trachomatis$  clone CtL2gam-26.
- SEQ ID NO: 276 is the determined DNA sequence for the  $\it C$ . 25  $\it trachomatis$  clone CtL2gam-24.
  - SEQ ID NO: 277 is the determined DNA sequence for the C. trachomatis clone CtL2gam-23.
    - SEQ ID NO: 278 is the determined DNA sequence for the  $\it C$ .  $\it trachomatis$  clone CtL2gam-21.
- 30 SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis clone CtL2gam-18.
  - SEQ ID NO: 280 is the determined DNA sequence for the  $\it C$ .  $\it trachomatis$  clone CtL2gam-17.
- SEQ ID NO: 281 is a first determined DNA sequence for the C. 35 trachomatis clone CtL2gam-15 representing the 5' end.

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SEQ ID NO: 282 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 3' end.

SEQ ID NO: 283 is the determined DNA sequence for the  $\it C$ . trachomatis clone CtL2gam-13.

5 SEQ ID NO: 284 is the determined DNA sequence for the C. trachomatis clone CtL2gam-10.

SEQ ID NO: 285 is the determined DNA sequence for the  $\it C.$  trachomatis clone CtL2gam-8.

SEQ ID NO: 286 is a first determined DNA sequence for the C.

10 trachomatis clone CtL2gam-6 representing the 5' end.

SEQ ID NO: 287 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 3' end.

SEQ ID NO: 288 is the determined DNA sequence for the  $\it C$ .  $\it trachomatis$  clone CtL2gam-5.

15 SEQ ID NO: 289 is the determined DNA sequence for the C. trachomatis clone CtL2gam-2.

SEQ ID NO: 290 is the determined DNA sequence for the C. trachomatis clone CtL2gam-1.

SEQ ID NO: 291 is the determined full-length DNA sequence for the C.

20 pneumoniae homologue of the CT529 gene.

SEQ ID NO: 292 is the predicted full-length amino acid sequence for the C. pneumoniae homologue of the CT529 gene.

SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the SKB vaccine vector.

25 SEQ ID NO: 294 is the amino acid sequence of an open reading frame of clone CT603.

SEQ ID NO: 295 is the amino acid sequence of a first open reading frame of clone CT875.

SEQ ID NO: 296 is the amino acid sequence of a second open reading 30 frame of clone CT875.

SEQ ID NO: 297 is the amino acid sequence of a first open reading frame of clone CT858.

SEQ ID NO: 298 is the amino acid sequence of a second open reading frame of clone CT858.

35 SEQ ID NO: 299 is the amino acid sequence of an open reading frame of clone CT622.

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10 L2 rCt529c1-125.

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SEO ID NO: 300 is the amino acid sequence of an open reading frame of clone CT610.

SEQ ID NO: 301 is the amino acid sequence of an open reading frame of clone CT396.

SEO ID NO: 302 is the amino acid sequence of an open reading frame of clone CT318.

SEQ ID NO: 304 is the amino acid sequence for C. trachomatis, serovar L2 rCt529c1-125 having a modified N-terminal sequence (6-His tag).

SEO ID NO: 305 is the amino acid sequence for C. trachomatis, serovar

SEQ ID NO: 306 is the sense primer used in the synthesis of the PmpA(N-term) fusion protein.

SEO ID NO: 307 is the antisense primer used in the synthesis of the PmpA(N-term) fusion protein.

SEO ID NO: 308 is the DNA sequence encoding the PmpA(N-term)

fusion protein.

SEQ ID NO: 309 is the amino acid sequence of the PmpA(N-term) fusion protein.

SEO ID NO: 310 is the sense primer used in the synthesis of the 20 PmpA(C-term) fusion protein.

SEO ID NO: 311 is the antisense primer used in the synthesis of the PmpA(C-term) fusion protein.

SEO ID NO: 312 is the DNA sequence encoding the PmpA(C-term) fusion protein.

25 SEO ID NO: 313 is the amino acid sequence of the PmpA(C-term) fusion protein.

SEO ID NO: 314 is the sense primer used in the synthesis of the PmpF(N-term) fusion protein.

SEQ ID NO: 315 is the antisense primer used in the synthesis of the PmpF(N-term) fusion protein.

SEO ID NO: 316 is the DNA sequence encoding the PmpF(N-term)

fusion protein. SEO ID NO: 317 is the amino acid sequence of the PmpF(N-term)

fusion protein.

35 SEQ ID NO: 318 is the sense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEQ ID NO: 319 is the antisense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEO ID NO: 320 is the DNA sequence encoding the PmpF(C-term) fusion protein.

5 SEO ID NO: 321 is the amino acid sequence of the PmpF(C-term) fusion protein.

SEO ID NO: 322 is the sense primer used in the synthesis of the PmpH(N-term) fusion protein.

SEO ID NO: 323 is the antisense primer used in the synthesis of the PmpH(N-term) fusion protein.

SEO ID NO: 324 is the DNA sequence encoding the PmpH(N-term) fusion protein.

SEO ID NO: 325 is the amino acid sequence of the PmpH(N-term) fusion protein.

15 SEO ID NO: 326 is the sense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 327 is the antisense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 328 is the DNA sequence encoding the PmpH(C-term) fusion protein. SEO ID NO: 329 is the amino acid sequence of the PmpH(C-term)

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fusion protein. SEO ID NO: 330 is the sense primer used in the synthesis of the

PmpB(1) fusion protein.

SEO ID NO: 331 is the antisense primer used in the synthesis of the 25 PmpB(1) fusion protein.

SEQ ID NO: 332 is the DNA sequence encoding the PmpB(1) fusion protein.

SEO ID NO: 333 is the amino acid sequence of the PmpB(1) fusion

30 protein.

> SEO ID NO: 334 is the sense primer used in the synthesis of the PmpB(2) fusion protein.

> SEO ID NO: 335 is the antisense primer used in the synthesis of the PmpB(2) fusion protein.

35 SEO ID NO: 336 is the DNA sequence encoding the PmpB(2) fusion protein.

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SEQ ID NO: 337 is the amino acid sequence of the PmpB(2) fusion protein.

SEQ ID NO: 338 is the sense primer used in the synthesis of the PmpB(3) fusion protein.

5 SEQ ID NO: 339 is the antisense primer used in the synthesis of the PmpB(3) fusion protein.

SEQ ID NO: 340 is the DNA sequence encoding the PmpB(3) fusion protein.

SEQ ID NO: 341 is the amino acid sequence of the PmpB(3) fusion

10 protein.

protein.

protein.

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SEQ ID NO: 342 is the sense primer used in the synthesis of the PmpB(4) fusion protein.

SEQ ID NO: 343 is the antisense primer used in the synthesis of the PmpB(4) fusion protein.

SEQ ID NO: 344 is the DNA sequence encoding the PmpB(4) fusion

SEQ ID NO: 345 is the amino acid sequence of the PmpB(4) fusion protein.

SEQ ID NO: 346 is the sense primer used in the synthesis of the 20 PmpC(1) fusion protein.

SEQ ID NO: 347 is the antisense primer used in the synthesis of the PmpC(1) fusion protein.

SEQ ID NO: 348 is the DNA sequence encoding the  $\mbox{PmpC}(1)$  fusion protein.

25 SEQ ID NO: 349 is the amino acid sequence of the PmpC(1) fusion protein.

SEQ ID NO: 350 is the sense primer used in the synthesis of the PmpC(2) fusion protein.

SEQ ID NO: 351 is the antisense primer used in the synthesis of the 30 PmpC(2) fusion protein.

SEQ ID NO: 352 is the DNA sequence encoding the PmpC(2) fusion

SEQ ID NO: 353 is the amino acid sequence of the PmpC(2) fusion protein.

35 SEQ ID NO: 354 is the sense primer used in the synthesis of the PmpC(3) fusion protein. WO 01/40474 PCT/US00/32919

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SEQ ID NO: 355 is the antisense primer used in the synthesis of the PmpC(3) fusion protein.

SEQ ID NO: 356 is the DNA sequence encoding the PmpC(3) fusion protein.

SEQ ID NO: 357 is the amino acid sequence of the PmpC(3) fusion protein.

# DESCRIPTION OF THE FIGURES

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Fig. 1 illustrates induction of INF-γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

10 Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.

Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).

15 Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with C. trachomatis SWIB protein.

Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.

Fig. 6 illustrates the 5' and 3' primer sequences designed from C. pneumoniae which were used to isolate the SWIB and S13 genes from C. pneumoniae.

Figs. 7A and 7B show induction of IFN- $\gamma$  from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to *C. trachomatis* and *C. pneumonia* upon activation by monocyte-derived dendritic cells expressing chlamydial proteins.

Fig. 8 shows the identification of T cell epitopes in Chlamydial 25 ribosomal \$13 protein with T-cell line TCL 8 EB/DC.

Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against *C. pnuemoniae*-infected dendritic cells to recombinant *C. pneumoniae*-SWIBprotein, but not *C. trachomatis* SWIB protein.

Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.

Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

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# DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a Chlamydia antipen, or a variant thereof.

In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native Chlamydla antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

20 The term "polynucleotide(s)," as used herein, means a single or doublestranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA
and corresponding RNA molecules, including HnRNA and mRNA molecules, both
sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant
DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule
contains introns and corresponds to a DNA molecule in a generally one-to-one manner.
An mRNA molecule corresponds to an HnRNA and DNA molecule from which the
introns have been excised. A polynucleotide may consist of an entire gene, or any
portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the
corresponding polynucleotide, and the definition of "polynucleotide" therefore includes
30 all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (*i.e.*, generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and

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most preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed.. Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include 5 screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigenspecific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well 10 known techniques. An immunogenic portion of a native Chlamvdia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, 125I-labeled Protein A. 20

Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

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The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such

that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine. isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, 25 threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including 35 the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example,

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a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to 5 enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished. 10 relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotidedirected site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences "Substantial homology," as used herein, refers to specifically recited herein. polynucleotide sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, 25 encode a polypeptide that is the same as a polypeptide of the present invention.

Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences 35 are optimally aligned.

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Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A 5 model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) 10 Fast and sensitive multiple sequence alignments on a microcomputer CABIOS 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy -15 the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks Proc. Natl. Acad., Sci. USA 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL.

Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), 25 or by inspection.

One illustrative example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) Nuc. Acids Res. 25:3389-3402 and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 30 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.nebi.nlm.nih.gov/) In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be

used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either 5 sequence is reached. The BLAST algorithm parameters W. T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

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Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or 15 less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention provides polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% or more sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence 25 identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two polynucleotide sequences by taking into account codon degeneracy, amino acid 30 similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides or polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed 35 herein. For example, polynucleotides and polypeptides encompassed by this invention may comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the disclosed sequences, as well as all intermediate lengths therebetween. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, etc.; 21, 22, 23, etc.; 30, 31, 32, etc.; 50, 51, 52, 53, etc.; 100, 101, 102, 103, etc.; 150, 151, 152, 153, etc.; including all integers through the 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least 20 one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the 25 others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the 30 group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamvdia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived 35 dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and Chlamydia pneumoniae. The antigens may thus be

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employed in a vaccine for both C. trachomatis genital tract infections and for C. pneumonia infections. Further characterization of these Chlamydia antigens from Chlamydia trachomatis and Chlamydia pneumonia to determine the extent of crossreactivity is provided in Example 6. Additionally, Example 4 describes cDNA 5 fragments (SEQ ID NO: 15, 16 and 33) isolated from C. trachomatis which encode proteins (SEO ID NO: 17-19 and 32) capable of stimulating a Chlamydia-specific murine CD8+ T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, 10 polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamvdia genomic or cDNA expression library by screening with a Chlamvdiaspecific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein.. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequencespecific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a Chlamydiainfected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. See Edman and Berg, Eur. J. Biochem. 80:116-132, 1967.

Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate Chlamydia cDNA or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a 35 screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold

Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated 5 probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is 10 size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with 32P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are 25 then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially 30 available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp, Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may

35 be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

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One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the 5 known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the 20 promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoterprimer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly 25 synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

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Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-5 directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamvdial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (i.e., an 15 antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a Chlamydial protein. Antisense technology can be used to control gene expression through triplehelix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (see Gee et al., In Huber and Carr, Molecular and Immunologic Approaches, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (e.g., promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

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Any polynucleotide may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking 35 sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional

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bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. 5 example, a polynucleotide may be cloned into any of a variety of cloning vectors. including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or 10 more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the 15 commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

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As noted above, immunogenic portions of Chlamydia antigens may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen 25 for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a Chlamydia antigen generates at least about 20%, and 30 preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of Chlamydia antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific 35 mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

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Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher 15 eukaryotic cells. Preferably, the host cells employed are E. coli, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

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In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the 20 polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at 25 least one polypeptide as described herein and an unrelated sequence, such as a known Chlamydial protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both 30 immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant 35 DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide 10 linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser 15 residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. 20 As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the 30 present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises

approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypentide with additional exogenous T-cell epitopes and to 5 increase the expression level in E. coli (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from Streptococcus pneumoniae, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; Gene 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of E. coli C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (see 20 Biotechnology 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the Cterminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In another embodiment, a Mycobacterium tuberculosis-derived Ra12 polynucleotide is linked to at least an immunogenic portion of a polynucleotide of this invention. Ral2 compositions and methods for their use inenhancing expression of heterologous polynucleotide sequences is described in U.S. Patent Application 60/158.585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a 30 Mycohacterium tuberculosis MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of M. tuberculosis. The nucleotide sequence and amino acid sequence of MTB32A have been described (U.S. Patent Application 60/158,585; see also, Skeiky et al., Infection and Immun. (1999) 67:3998-4007, incorporated herein by reference. In one embodiment, 35 the Ra12 polypeptide used in the production of fusion polypeptides comprises a Cterminal fragment of the MTB32A coding sequence that is effective for enhancing the expression and/or immunogenicity of heterologous Chlamydial antigenic polypeptides with which it is fused. In another embodiment, the Ra12 polypeptide corresponds to an approximately 14 kD. C-terminal fragment of MTB32A comprising some or all of amino acid residues 192 to 323 of MTB32A.

5 Recombinant nucleic acids, which encode a fusion polypeptide comprising a Ra12 polypeptide and a heterologous Chlamydia polypeptide of interest, can be readily constructed by conventional genetic engineering techniques. Recombinant nucleic acids are constructed so that, preferably, a Ra12 polynucleotide sequence is located 5' to a selected heterologous Chlamydia polynucleotide sequence. It may also be appropriate to place a Ra12 polynucleotide sequence 3' to a selected heterologous polynucleotide sequence or to insert a heterologous polynucleotide sequence into a site within a Ra12 polynucleotide sequence.

In addition, any suitable polynucleotide that encodes a Ral2 or a portion or other variant thereof can be used in constructing recombinant fusion polynucleotides 15 comprising Ra12 and one or more Chlamydia polynucleotides disclosed herein. Preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide.

20 Ra12 polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ral2 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a 25 fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

In another aspect, the present invention provides methods for using one 30 or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

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In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other Chlamydia antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

10 Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve 20 the use of a non-pathogenic (defective) virus. Techniques for incorporating polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by 25 Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting mojety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target 30 specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked

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polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune response-modifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-Chlamydia effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-diotypic antibodies (as in U.S. Patent No. 4.918.164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunetherapy is to grow immune T-cells in vitro. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition in vivo are well known in the art. These in vitro culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting

cells may be transfected or transduced with a polynucleotide sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipid-mediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," Immunological Reviews, 157:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

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Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigenspecific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al.* (*Crit. Rev. Oncol. Hematol.*, 22(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as Isolex™ System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the

35 polypeptides disclosed herein can be cloned, expanded, and transferred into other
vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may

be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-5 independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, Immunological Reviews, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated in vitro for autologous transplant back into the same patient.

20 Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (i.e., vaccines). Alternatively, a pharmaceutical composition may comprise an antigen-presenting cell (e.g., a dendritic cell) transfected with a Chlamydial polynucleotide such that the antigen presenting cell expresses a Chlamvdial polypeptide. Pharmaceutical compositions comprise one or more such 25 compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic

portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated in situ. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, Crit. Rev. Therap. Drug Carrier Systems 15:143-198, 1998, and references cited therein.

O Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, adenovirus, baculovirus, togavirus, bacteriophage, and the like), which often involves the use of a non-pathogenic (defective), replication competent virus.

For example, many viral expression vectors are derived from viruses of the retroviridae family. This family includes the murine leukemia viruses, the mouse mammary tumor viruses, the human foamy viruses, Rous sarcoma virus, and the immunodeficiency viruses, including human, simian, and feline. Considerations when designing retroviral expression vectors are discussed in Comstock et al. (1997).

Excellent murine leukemia virus (MLV)-based viral expression vectors

have been developed by Kim et al. (1998). In creating the MLV vectors, Kim et al. found that the entire gag sequence, together with the immediate upstream region, could be deleted without significantly affecting viral packaging or gene expression. Further, it was found that nearly the entire U3 region could be replaced with the immediately-early promoter of human cytomegalovirus without deleterious effects. Additionally, MCR and internal ribosome entry sites (IRES) could be added without adverse effects. Based on their observations, Kim et al. have designed a series of MLV-based expression vectors comprising one or more of the features described above.

As more has been learned about human foamy virus (HFV), characteristics of HFV that are favorable for its use as an expression vector have been 35 discovered. These characteristics include the expression of pol by splicing and start of

translation at a defined initiation codon. Other aspects of HFV viral expression vectors are reviewed in Bodem et al. (1997).

Murakami et al. (1997) describe a Rous sarcoma virus (RSV)-based replication-competent avian retrovirus vectors, IR1 and IR2 to express a heterologous gene at a high level. In these vectors, the IRES derived from encephalomyocarditis virus (EMCV) was inserted between the env gene and the heterologous gene. The IR1 vector retains the splice-acceptor site that is present downstream of the env gene while the IR2 vector lacks it. Murakami et al. have shown high level expression of several different heterologous genes by these vectors.

Recently, a number of lentivirus-based retroviral expression vectors have been developed. Kafri et al. (1997) have shown sustained expression of genes delivered directly into liver and muscle by a human immunodeficiency virus (HIV)-based expression vector. One benefit of the system is the inherent ability of HIV to transduce non-dividing cells. Because the viruses of Kafri et al. are pseudotyped with vesicular stomatitis virus G glycoprotein (VSVG), they can transduce a broad range of tissues and cell types.

A large number of adenovirus-based expression vectors have been developed, primarily due to the advantages offered by these vectors in gene therapy applications. Adenovirus expression vectors and methods of using such vectors are the subject of a number of United States patents, including United States Patent No. 5,698,202, United States Patent No. 5,698,202, United States Patent No. 5,518,913, all incorporated herein by reference.

Additional adenoviral constructs are described in Khatri et al. (1997) and Tomanin et al. (1997). Khatri et al. describe novel ovine adenovirus expression vectors and their ability to infect bovine nasal turbinate and rabbit kidney cells as well as a range of human cell type, including lung and foreskin fibroblasts as well as liver, prostate, breast, colon and retinal lines. Tomanin et al. describe adenoviral expression vectors containing the T7 RNA polymerase gene. When introduced into cells containing a heterologous gene operably linked to a T7 promoter, the vectors were able to drive gene expression from the T7 promoter. The authors suggest that this system may be useful for the cloning and expression of genes encoding cytotoxic proteins.

Poxviruses are widely used for the expression of heterologous genes in mammalian cells. Over the years, the vectors have been improved to allow high expression of the heterologous gene and simplify the integration of multiple 35 heterologous genes into a single molecule. In an effort to diminish cytopathic effects and to increase safety, vaccinia virus mutant and other poxviruses that undergo abortive

infection in mammalian cells are receiving special attention (Oertli et al., 1997). The use of poxviruses as expression vectors is reviewed in Carroll and Moss (1997).

Togaviral expression vectors, which includes alphaviral expression vectors have been used to study the structure and function of proteins and for protein 5 production purposes. Attractive features of togaviral expression vectors are rapid and efficient gene expression, wide host range, and RNA genomes (Huang, 1996). Also, recombinant vaccines based on alphaviral expression vectors have been shown to induce a strong humoral and cellular immune response with good immunological memory and protective effects (Tubulekas et al., 1997). Alphaviral expression vectors and their use are discussed, for example, in Lundstrom (1997).

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In one study, Li and Garoff (1996) used Semliki Forest virus (SFV) expression vectors to express retroviral genes and to produce retroviral particles in BHK-21 cells. The particles produced by this method had protease and reverse transcriptase activity and were infectious. Furthermore, no helper virus could be detected in the virus stocks. Therefore, this system has features that are attractive for its use in gene therapy protocols.

Baculoviral expression vectors have traditionally been used to express heterologous proteins in insect cells. Examples of proteins include mammalian chemokine receptors (Wang et al., 1997), reporter proteins such as green fluorescent protein (Wu et al., 1997), and FLAG fusion proteins (Wu et al., 1997; Koh et al., 1997). 20 Recent advances in baculoviral expression vector technology, including their use in virion display vectors and expression in mammalian cells is reviewed by Possee (1997). Other reviews on baculoviral expression vectors include Jones and Morikawa (1996) and O'Reilly (1997).

25 Other suitable viral expression systems are disclosed, for example, in Fisher-Hoch et al., Proc. Natl. Acad. Sci. USA 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; 30 Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219, 1994; Kass-Eisler et al., Proc. Natl. Acad. Sci. USA 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. In other systems, the DNA may be 35 introduced as "naked" DNA, as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

It will be apparent that a vaccine may comprise a polynucleotide and/or a polypeptide component, as desired. It will also be apparent that a vaccine may contain 5 pharmaceutically acceptable salts of the polynucleotides and/or polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts). While any suitable carrier known to those of ordinary 10 skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as 15 subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier. such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable 20 microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium tuberculosis derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant

and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2. -7, or -12, may also be used as adiuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of 10 the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-7, TNFa, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman. Ann. Rev. Immunol. 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type 20 response include, for example, a combination of monophosphoryl lipid A, preferably 3de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent Nos. 4.436,727; 4.877,611; 4.866,034 and 4.912,094). CpG-containing oligonucleotides (in 25 which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., Science 273:352, 1996. Another preferred adjuvant is a saponin, preferably OS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of OS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the OS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

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Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa Corporation; Seattle, WA), RC-529 (Corixa 5 Corporation; Seattle, WA) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties

Any vaccine provided herein may be prepared using well known to methods that result in a combination of antigen, immunostimulant and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., Vaccine 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

20 Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-coglycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid bydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see e.g., U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets Chlamydia-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the

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antigen, to improve activation and/or maintenance of the T cell response, to have antiChlamydia effects per se and/or to be immunologically compatible with the receiver
(i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety
of biological fluids and organs, and may be autologous, allogeneic, syngeneic or
xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-15 surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated ex νίνο by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature"

30 cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature behotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and

class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a Chlamydial protein (or portion or other variant thereof) such that the Chlamydial 5 polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In 10 vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA 15 (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide. 20

Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intranuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from Chlamydial infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 100 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

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While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable of microspheres are disclosed, for example, in U.S. Patent Nos. 4.897.268 and 5.075.109.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in 15 preexisting immune responses to a Chlamydial protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

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As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as 30 described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to Chlamydia antigens which may be indicative of Chlamydia-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another

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component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with Chlamydia. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more 5 polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, 10 which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled 15 with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

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The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present 30 invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable 35 buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general,

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contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 µg, and preferably about 100 ng. is sufficient to bind an adequate amount of antigen.

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Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, 0.00). Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13.

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20<sup>TM</sup> (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween  $20^{\text{TM}}$ . Detection reagent may then be added to the solid support. An appropriate detection reagent is any

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compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.e., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

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The detection reagent is then incubated with the immobilized antibodypolypeptide complex for an amount of time sufficient to detect the bound antibody. An
appropriate amount of time may generally be determined from the manufacturer's
instructions or by assaying the level of binding that occurs over a period of time.

Unbound detection reagent is then removed and bound detection reagent is detected
using the reporter group. The method employed for detecting the reporter group
depends upon the nature of the reporter group. For radioactive groups, scintillation
counting or autoradiographic methods are generally appropriate. Spectroscopic
methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin
may be detected using avidin, coupled to a different reporter group (commonly a
radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally
be detected by the addition of substrate (generally for a specific period of time),
followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamvdia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid 25 support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above 30 the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true 35 positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off

value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to 5 minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as 10 nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the 15 strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-Chlamvdia antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to 30 be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

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The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a Chlamydial protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically

bind" to a Chlamydial protein if it reacts at a detectable level (within, for example, an ELISA) with a Chlamydial protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability 5 to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 103 10 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a Chlamydial infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a Chlamydial protein will generate a signal indicating the presence of a Chlamydial 15 infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, sputum urine and/or tissue biopsies ) from patients with and without Chlamydial infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and 30 Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is 35 initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen

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without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest 10 may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as 15 described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, 20 aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in for example, an affinity chromatography step.

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Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane,

Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include <sup>50</sup>Y, <sup>123</sup>I, <sup>133</sup>I, <sup>185</sup>Re, <sup>188</sup>Re, <sup>211</sup>At, and <sup>212</sup>Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction

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of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

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It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of Chlamydia antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions

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thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify Chlamydia-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The 5 presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

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As used herein, the term "oligonucleotide primcr/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 15 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect Chlamydia-specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

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## EXAMPLE 1

## ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-y in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

A randomly sheared genomic library of Chlamydia trachomatis LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 μl of RPMI 10% FBS. 10 μl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free E. coli and Chlamydia-specific T cells were added. Positive E. coli pools were identified by determining IFN-γ production and proliferation of the T cells in response to the pools.

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Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEO ID NO: 25 1-4. respectively. Clone 1-B1-66 is approximately in region 536690 of the C. trachomatis genome (NCBI C. trachomatis database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the C. trachomatis genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published 35 sequence for S13 ribosomal protein from Chlamydia trachomatis (Gu, L. et al. J. Bacteriology, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and 10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEO ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of Chlamydia trachomatis LGV II described above.

5 A Chlamydia-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of Chlamydia trachomatis LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the Chlamydia-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

In further studies involving clone 4C9-18 (SEQ ID NO: 21), the fulllength amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from C. trachomatis (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

To further characterize the open reading frame containing the T cell 20 stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the NdeI/EcoRI site of the pET17b vector (Novagen, Madison, WI). referred to as clone 4C9-18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into E. coli. Selective induction of the transformed E. coli with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, E. coli expressing the 26 kDa protein 30 were titered onto 1 x 104 monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 104 T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-γ in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a Chlamvdia-specific T-cell response against the lipoamide dehydrogenase sequence.

Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the TCT-1 T-cell line by standard proliferation assays.

Subsequent studies to identify additional Chlamydia trachomatis antigens using the above-described CD4+ T-cell expression cloning technique yielded 5 additional clones. The TCT-1 and TCL-8 Chlamydia-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the Chlamydia trachomatis LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to Chlamydia pnuemoniae. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-15 21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pnuemoniae.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEO ID NO: 61), identified using the TCT-3 cell line, has two partial open reading frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a 25 complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, 30 (SEO ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line,

so contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire

ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone

22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEQ ID NO: 81), identified using the TCT-3 cell line, contains a 2.085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the 5 complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 10 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp 2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using 15 the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as part of the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SvcE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, 20 (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

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Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of Chlamydia trachomatis 25 contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpl. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a Chlamydial infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

Based on the Chlamydia trachomatis LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpl. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, 35 London, England). Specifically, PmpC was subcloned into the JAL vector using the 5'

Oligo GAT AGG CGC GCC GCA ATC ATG AAA TIT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the 5 JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine 10 vector following PCR amplification of the gene using the following oligos: 5' oligo-TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C (SEQ ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEO ID NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC 15 (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindlII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEO ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEO ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG ( SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned 30 into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli 35 BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel

chromatographic methodology provided by Novagen. Several of the genes encoding

the recombinant proteins, as described below, lack the native signal sequence to facilitate expression of the protein. Full-length protein expression of pmpC was accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the 5 signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CCA TCA CGA GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy 10 terminus portion of the gene, pmpC-carboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' Nhel/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC 15 CTT AGA ATG TCA TAC GAG CAC CGC AG (SEO ID NO: 210). PmpD was also expressed as two overlapping proteins. The pmpD-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a six-20 histidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CCA TCA CGG GTT AGC (SEQ ID NO: 211), and the 3' oligo- CAG AGG TAC CTC AGC TCC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEO ID NO: 192). For expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG TGG (SEO ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191). The pmpE insert also lacks the native signal sequence. PCR amplification of the gene under conditions well known in the art was performed using

the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and the 3' oligo-CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short 5 nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. The expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence 10 described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEO ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo-CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional amino end. at the 20 amino acid sequence MASMTGGQQNGRDSSLVPHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the 25 following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' NheI/3' SpeI cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st 30 amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant – CT603 (the CT603 ORF is a homolog of CPn0778 from C. pmemoniae). The TSA open reading frame in clone 14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert

was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 10 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 15 951 bp insert that contains part of the ORF for DT431, encoding for clpP 1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). 20 Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was shown to be part of the dnaK gene CT396 Clone 17-C10-31 (SEQ ID NO: 114), identified by the TCT-3 cell line, contains a 976 bp insert. This clone contains part of 25 the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 (SEO ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase(gyrA 2)) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary strand. Clone 22-B3-53 (SEO ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1

vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first 5 determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEO ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID 10 NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); 15 CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional Chlamydia trachomatis antigens were identified by 20 serological expression cloning. These studies used sera pooled from several Chlamydia-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a Chlamydial infection, that is a mucosal humoral immune response. The following immunoreactive 25 clones were characterized and the inserts containing Chlamydia genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ ID NO: 289), CTL2gam-5 (SEQ ID NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ 30 ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 35 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence

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representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

#### EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y

5

PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

The ability of recombinant *Chlamydia trachomatis* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. trachomatis* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 15 50 µg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 µg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 µl, 50 µl of medium is removed from each well for determination of IFN-y levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 m using 570 mm as a reference wavelength. Fractions that result in both replicates giving

an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 5 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-γ production in a Chlamydia-specific T cell line used to screen a genomic library of C. trachomatis LGV II.

Further studies have identified a *C. trachomatis*-specific T-cell epitope

in the ribosomal S13 protein. Employing standard epitope mapping techniques well
known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were
identified with a *Chlamydia*-specific T-cell line from donor CL-8 (T-cell line TCL-8
EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100%
identical with the corresponding *C. pneumoniae* sequence, explaining the crossreactivity of the T-cell line to recombinant *C. trachomatis*- and *C. pneumoniae*-rS13.
The response to the second peptide rS13 56-75 (SEQ ID NO: 108) is *C. trachomatis*specific, indicating that the rS13 response in this healthy asymptomatic donor was
elicited by exposure to *C. trachomatis* and not to *C. pneumoniae*, or any other microbial
infection.

As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), 20 identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay 25 previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10<sup>4</sup> TCP-21 T-cells in the presence of 1 x 10<sup>4</sup> monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes 30 CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two 35 (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed 5 as described above, except that only peptides from C. trachomatis were tested. The Tcells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of C. trachomatis

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEO ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope mapping immunoassays were performed, as described above, to 15 further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of C. trachomatis serovar LGVII.

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# EXAMPLE 3

# PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be 25 attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried trifluoroacetic following cleavage mixture: using the acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then 30 be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

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# EXAMPLE 4

# ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

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A genomic library of Chlamydia trachomatis LGV II was constructed by limited digests using BamHI, BgIII, BstYi and Mbol restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helperfree Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene 15 Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The Chlamydia library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2<sup>4</sup> restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-y production using Elispot analysis (see Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the Chlamydia-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in an additional positive clone, which contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID

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NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of Chlamydia trachomatis, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as 10 CT529 and/or the Cap1 gene) from serovar L2 was obtained by PCR amplification using 5'-ttttgaagcatgtgagtgatatg (forward) (SEQ ID NO: 159) and 5'-ttagagaatttgaagaattcaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified C. trachomatis L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the 15 EcoRI site of pBIB-KMS, a derivative of pBIB-KS for expression. The Chlamydia pnuemoniae homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 105 IFU, essentially as described (Denamur, E., 20 C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEO ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the 25 corresponding predicted amino acid sequence provided in SEQ ID NO: 129); G; (SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEQ ID NO: 124, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 125); L1 (SEO ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the 30 corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'- ggtataatatctctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaaggcatttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia. CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-cettacacagtectgetgac (SEQ ID NO: 165) and a reverse primer 3'-gtttcegggecctcacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NII), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping 15 peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2d restricted target cells. In this assay, aliquots of P815 cells (H2<sup>d</sup>) were labeled at 37° C for one hour with 100 uCi of 51Cr in the presence or absence of 1 µg/ml of the indicated 20 peptides. Following this incubation, labeled P815 cells were washed to remove excess 51Cr and peptide, and subsequently plated in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were added at the indicated effector:target ratios. Following a 4 hour incubation, supernatants were harvested and measured by gamma-counter for release of 51Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEQ ID NO: 138-156 were synthesized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame. As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-30 147, SEQ ID NO: 139 ) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEO ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2d (Kd and Ld) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was 35 found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no

proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEQ ID NO: 15) defines a gene which encompasses an antigen from *Chlamydia* capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against *Chlamydia*.

To confirm these results and to further map the epitope, truncated peptides (SEQ ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEQ ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the Chlamydia-specific T-cells.

Sequence alignments of Cap1 (CT529) from selected serovars of C.

trachomatis (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the Chlamydia specific T-cells was compared. Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a <sup>31</sup>Cr release assay, as described above. The Chlamydia-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1<sub>139-147</sub> is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2\*) cells were infected with *C. trachomatis* serovar L2 and tested to determine whether these cells are recognized by a 25 CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with *C. trachomatis*. To determine if infection with *C. trachomatis* primes a Cap1#139-147 epitope-specific T-cell response, mice were

infected i.p. with 10<sup>8</sup> IFU of *C. trachomatis* serovar I.2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard <sup>51</sup>Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar I.2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

Studies were performed demonstrating that Ct529 (referred to herein as 15 Cap-1) localizes to the inclusion membrane of *C. trachomatis*-infected cells and is not associated with elementary bodies or reticulate bodies. As described above, Cap-1 was identified as a product from *Chlamydia* that stimulates CD8+ CTL. These CTL are protective in a murine model of infection, thus making Cap-1 a good vaccine candidate. Further, since these CTL are MHC-1 restricted, the Cap-1 gene must have access to the cytosol of infected cells, which may be a unique characteristic of specific *Chlamydial* gene products. Therefore, determination of the cellular localization of the gene products would be useful in characterizing Cap-1 as a vaccine candidate. To detect the intracellular localization of Cap-1, rabbit polyclonal antibodies directed against a recombinant polypeptide encompassing the N-terminal 125 amino acids of Cap-1 (SEQ ID NO: 305, with the amino acid sequence including the N-terminal 6-this tag provided in SEQ ID NO: 304) were used to stain McCoy cells infected with *Chlamydiae*.

Rabbit-anti-Cap-1 polyclonal antibodies were obtained by hyperimmunization of rabbits with a recombinant polypeptide, rCt529e1-125 (SEQ ID NO:
305) encompassing the N-terminal portion of Cap-1. Recombinant rCt529e1-125
30 protein was obtained from *E. coli* transformed with a pET expression plasmid (as
described above) encoding the nucleotides 1-375 encoding the N-terminal 1-125 amino
acids of Cap-1. Recombinant protein was purified by Ni-NTA using techniques well
known in the art. For a positive control antiserum, polyclonal antisera directed against
clementary bodies were made by immunization of rabbits with purified *C. trachomatis*35 elementary bodies (Biodesign, Sacco, Maine). Pre-immune sera derived from rabbits
prior to immunization with the Cap-1 polypeptide was used as a negative control.

Immunocytochemistry was performed on McCoy cell monolayers grown on glass coverslips inoculated with either C. trachomatis serovar L2 or C. psitacci, strain 6BC, at a concentration of 10<sup>6</sup> IFU (Inclusion Forming Units) per ml. After 2 hours, medium was aspirated and replaced with fresh RP-10 medium supplemented 5 with cycloheximide (1.0 μg/ml). Infected cells were incubated at in 7% CO<sub>2</sub> for 24 hours and fixed by aspirating medium, rinsing cells once with PBS and methanol fixation for 5 minutes. For antigen staining, fixed cell monolayers were washed with PBS and incubated at 37°C for 2 hours with 1:100 dilutions of specific or control antisera. Cells were rinsed with PBS and incubated for 1 hour with fluorescein isothiocyanate (FITC)-labeled, anti-rabbit IgG (KPL, Gaithersburg) and stained with Evans blue (0.05%) in PBS. Fluorescence was observed with a 100X objective (Zeiss erifluorescence microscope), and photographed (Nikon UFX-11A camera).

Results from this study show Cap-1 localizes to the inclusion membrane of C. trachomatis-infected cells. Cap-1 specific antibody labeled the inclusion membranes of C. trachomatis-infected cells, but not Chlamydial elementary bodies contained in these inclusions or released by the fixation process. Conversely, the anti-elementary body antibody clearly labeled the bacterial bodies, not only within the inclusions, but those released by the fixation process. Specificity of the anti-Cap-1 antibody is demonstrated by the fact that it does not stain C. psittaci-infected cells.

20 Specificity of the Cap-1 labeling is also shown by the absence of reactivity in pre-immune sera. These results suggest that Cap-1 is released from the bacteria and becomes associated with the Chlamydial inclusion membrane. Therefore, Cap-1 is a gene product which may be useful for stimulating CD8+ T cells in the development of a vaccine against infections caused by Chlamydia.

The relevance of the Cap-1 gene as a potential CTL antigen in a vaccine against Chlamydia infection is further illustrated by two additional series of studies. First, CTL specific for the MHC-1 epitope of Cap-1 CT529 #138-147 peptide of C. trachomatis (SEQ ID NO: 144) have been shown to be primed to a high frequency during natural infection. Specifically, Balb/C mice were inoculated with 10<sup>6</sup> I.F.U. of 30 C. trachomatis, serova L2. After 2 weeks, spleens were harvested and quantified by Elispot analysis for the number of IFN-γ secreting cells in response to Cap-1 #138-147 peptide-pulsed antigen presenting cells. In two experiments, the number of IFN-γ secreting cells in 10<sup>3</sup> splenocytes was about 1% of all CD8+ T-cells. This high frequency of responding CD8+ CTL to the MHC-1 epitope (Cap-1 CT529 #138-147 peptide) suggest that Cap-1 is highly immunogenic in infections.

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Results from a second series of studies have shown that the Cap-I protein is almost immediately accessible to the cytosol of the host cell upon infection. This is shown in a time-course of Cap-I CT529 #138-147 peptide presentation. Briefly, 3T3 cells were infected with C. trachomatis serovar L2 for various lengths of time, and 5 then tested for recognition by Cap-I CT529 #138-147 peptide-specific CTL. The results show that C. trachomatis-infected 3T3 cells are targeted for recognition by the antigen-specific CTL after only 2 hours of infection. These results suggest that Cap-I is an early protein synthesized in the development of C. trachomatis elementary bodies to reticulate bodies. A CD8+ CTL immune response directed against a gene product 10 expressed early in infection may be particularly efficacious in a vaccine against Chlamudia infection.

# EXAMPLE 5

GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH

CHLAMYDIA ANTIGENS

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Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also 20 referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEO ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ T-cell responses. DNA 25 immunizations were intradermal at the base of the tail and polypeptide immunizations were administered by subcutaneous route. Results from standard 3H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEQ ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-30 4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with 10 ug purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5)

formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, Cancer Research, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an Chlamydia-specific immune response.

In a further experiment, C3H mice were immunized at three separate

15 time points at the base of the tail with 10 µg of purified SWIB or S13 protein (C.

trachomatis, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham,
London, England). Antigen-specific antibody titers were measured by ELISA, showing
both polypeptides induced a strong IgG response, ranging in titers from 1 x10<sup>-4</sup> to 1 x10<sup>-2</sup>

3. The IgG1 and IgG2a components of this response were present in fairly equal
amounts. Antigen-specific T-cell proliferative responses, determined by standard <sup>3</sup>Hincorporation assays on spleen cells isolated from immunized mice, were quite strong
for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000
cpm above the negative control). The IFNy production was assayed by standard ELISA
techniques from supernatant from the proliferating culture. In vitro restimulation of the
culture with S13 protein induced high levels of IFNy production, approximately 25
ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein
also induced IFNy, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 µg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 µg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigen-specific antibody responses were determined by standard ELISA techniques. Antigen-specific IgG antibodies were present in the blood of SWIB-35 immunized mice, with titers ranging from 1 x10<sup>3</sup> to 1 x10<sup>4</sup>, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes,

as measured by IFNy production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 seroyar LGVII CTL epitope, defined by the CT529 10mer consensus pentide 5 (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 µg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered 10 intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a 15 standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigenspecific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

#### EXAMPLE 6

# EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes

Chlamydia trachomatis as well as Chlamydia pneumonia infected monocyte-derived dendritic cells, suggesting Chlamydia trachomatis and pneumonia may encode cross-reactive T-cell epitopes. To isolate the Chlamydia pneumonia genes homologous to Chlamydia trachomatis LGV II clones 1B1-66, also referred to as SWIB (SEQ ID NO: 30 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with C. pneumonia strain TWAR (CDC/CWL-029). After three days incubation, the C. pneumonia-infected HeLa cells were harvested, washed and resuspended in 200 µl water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the

3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The C. pneumonia-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and 5 subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from C. pneumonia were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively), a 15 kDa protein referred to as CpS13 (SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

# **EXAMPLE 7**

# INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

15 The ability of recombinant Chlamydia pneumoniae antigens to induce T cell proliferation and interferon-y production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. pneumoniae* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered 30 positive.

IFN-7 was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-7 (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for I hour at 35 room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at

room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-7 serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 5 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in 15 SEO ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEO ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic cells cultures were washed and 2.5 x 104 T cells (TCL-8) added 20 and allowed to incubate for an additional 72 hours. The amount of INF-y in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-y, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To 25 validate these results, the T cell epitope of C, trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the 30 TCL-8 line. The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEQ ID NO: 43) and C. trachomatis (SEO ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding peptides described above (SEQ ID NO; 41-43).

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Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-5 SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during in vivo *C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a *C. pneumoniae*-SWIB-specific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

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In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10<sup>4</sup> T-cells in the presence of 1 x 104 monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis 20 or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP 25 elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay 30 previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 104 TCP-21 T-cells in the presence of 1 x 104 monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes 35 CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-

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21 T-cell line also gave a proliferative response to the homologous *C. pneumoniae* peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the *C. trachomatis* peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between *C. trachomatis* and *C. pneumoniae*.

# **EXAMPLE 8**

#### IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES

10 AGAINST CHLAMYDIA ANTIGENS

The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. 15 trachomatis. To characterize the immune responses of normal donors against chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. 20 Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. pneumoniae elementary bodies. One donor, AD104, responded to recombinant C. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific response. Three out of 12 donors had a C. trachomatis-SWIB, but not a C. 25 pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae- S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a Tcell response in PBMC of normal study subjects.

TABLE I

# Immune response of normal study subjects against Chlamydia

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Donor	Sex	Chlamydia IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT lpdA	CT TSA
AD100	male	negative	++	+++	+	-	++	++	-	n.t.
AD104	female	negative	+++	++	-	-	-	++	-	n.t.
AD108	male	CP 1:256	++	++	+	+/-	+	+	+	n.t.
AD112	female	negative	++	++	+	-	+	-	+/-	n.t.
AD120	male	negative	-	+	-	-	-	-	-	n.t.
AD124	female	CP 1:128	++	++	-	-	-	-	-	n.t.
AD128	male	CP 1:512	+	++	-	-	++	+	++	-
AD132	female	negative	++	++	-	-	+	+	-	-
AD136	female	CP 1:128	+	++		-	+/-	-	-	-
AD140	male	CP 1:256	++	++	-	-	+	+	-	-
AD142	female	CP 1:512	++	++	-	-	+	+	+	-
AD146	female	negative	++	++			++	+	+	

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary
bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia
S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant
Chlamydia TSA protein. Values represent results from standard proliferation assays.
Proliferative responses were determined by stimulating 3 x 10<sup>5</sup> PBMC with 1 x 10<sup>4</sup>
monocyte-derived dendritic cells pre-incubated with the respective recombinant
antigens or elementary bodies (EB). Assays were harvested after 6 days with a <sup>3</sup>Hthymidine pulse for the last 18h.

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21: 21	imulation index	
+/-:	SI ~	4

+: SI > 4 ++: SI 10-30 +++: SI > 30

In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to *C. trachomatis* by stimulating T-cells with *C. trachomatis* LGV II elementary bodies as previously described. Although the study subject was exposed to *C. trachomatis*, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against *C. trachomatis*. As shown in Fig. 10, a primary *Chlamydia*-specific T-cell line derived from donor CT-10 responded to *C. trachomatis*-SWIB, but not *C. pneumoniae*-SWIB recombinant proteins, confirming the 15 exposure of CT-10 to *C. trachomatis*. Epitope mapping of the T-cell response to *C. trachomatis*-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEO ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C.

trachomatis patients. A summary of the patients' clinical profile and proliferative
responses to various C. trachomatis and C. pneumoniae elementary bodies and
recombinant proteins are summarized in Table II.

TABLE II

Proliferative response of C. trachomatis patients										
Patients	Clinical manifestation	IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT IpdA	CT TSA
CT-1	NGU	negative	+	+	-	-	++	++	++	+
CT-2	NGU	negative	++	++	-	-	+	+/-	-	-
CT-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	-	-	-
CT-5	BV	Ct 1:256 Cp 1:256	++	++	-	-	+	-	-	-
CT-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	-	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	-	-	+	+	+	-
CT-8	Not known	Not tested	++	++	-	-	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-	-	++	+	+	- '
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormal pap	Ct 1: 512	+++	+++	-	-	+++	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	-

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT=
Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary
bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia
S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant
Chlamydia TSA protein

Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10<sup>5</sup> PBMC with 1 x 10<sup>4</sup> monocyte-

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derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a 3H-thymidine pulse for the last 18 hours

# SI: Stimulation index

+/-:	SI ~	4
+:	SI >	4
++:	SI	10-30
+++•	SI>	30

10

25

Using the panel of asymptomatic (as defined above) study subjects and C. trachomatis patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from C. pneumoniae patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50  $\boldsymbol{\mu}$ g/ml gentamicin. Purified polypeptides, a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and S13, as well as . C. trachomatis lpdA and TSA are added in duplicate at concentrations of 0.5 to 10 µg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200  $\mu$ l, 50  $\mu$ l of medium is removed from each well for determination of IFN-y levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

Proliferative responses to the recombinant Chlamydiae antigens demonstrated that the majority of asymptomatic donors and C. trachomatis patients recognized the C. trachomatis S13 antigen (8/12) and a majority of the C. trachomatis patients recognized the C. pneumonia S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the C. pneumonia S13 antigen. Also, six out of twelve of the C. trachomatis patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of C. trachomatis. These results demonstrate that the C. trachomatis and C. pneumonia S13 antigen, C. trachomatis Swib antigen and the C. trachomatis lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to Chlamydia and an 35 immune response elicited against them. This implies these antigens may play a role in conferring protective immunity in a human host. In addition, the C. trachomatis and C. pneumonia S13 antigen is recognized equally well among the C. trachomatis patients,

therefore indicating there may be epitopes shared between *C. trachomatis* and *C. pneumonia* in the S13 protein. Table III summarizes the results of these studies.

TABLE III

	NORMAL DONORS	C.T. PATIENTS		
A. Antigen				
C.tSwib	3/12	0/12		
C.pSwib	0/12	0/12		
C.tS13	8/12	8/12		
C.pS13	4/12	8/12		
lpdA	4/12	6/12		
TSA	0/12	2/12		

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2.5

A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from asymptomatic donors and C. Cellular immune responses were measured by standard trachomatis patients. proliferation assays and IFN-γ, as described in Example 7. Specifically, the majority of 10 the antigens were in the form of single E. coli clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single E. coli clones were titered on 1 x 104 monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 104 T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after 15 four days with a standard 3H-thymidine pulse for the last 18 hours. Induction of IFN-γ was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the C. trachomatis antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form C. trachomatis patients. In addition, proliferative 20 responses were elicited from both the C. trachomatis patients and asymptomatic donors for the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83 clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences

from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

TABLE IV

Clone	C. t. Antigen (putative*)	TCL from Asymp. Donors	TCL from C. t. Patients	SEQ ID NO:
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	GROEL	1/2	. 4/4	111
22B3-53 (protein)	GROEL	1/2	4/4	111
15H2-76 (E. coli)	PMPD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	RS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	DNAK	0/2	2/4	59
21C7-8 (E. coli)	DNAK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

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# EXAMPLE 9

# PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal 10 inoculation that uses a human isolate containing a strain of Chlamydia psittaci (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as Chlamydia trachomatis, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by Chlamydia trachomatis in women. In the first experiment, C3H mice (4

inflammatory cells in the lumen of the oviduct

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95 mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were 5 progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct/ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary /oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated

mice showed no signs of tubal occlusion while negative control vaccinated groups had

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In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing C. trachomatis SWIB 20 DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of C. psittaci in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and 25 examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative controlimmunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 30 1.75 for the vaccinated group versus 3, 75 for the control.

In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID 35 NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia

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in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of C. psittaci or by injection of C. trachomatis serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for historathology. The 5 degree of inflammation was scored as described above. Scores attributed to each single oviduct/ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for 10 the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary /oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary /oviduct mean inflammation score of 8. The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection

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with C. psittaci.

### EXAMPLE 10

# PMP/Ra12 Fusion Proteins

Various Pmp/Ra12 fusion constructs were generated by first synthesizing PCR fragments of a Pmp gene using primers containing a Not I restriction 25 site. Each PCR fragment was then ligated into the Notl restriction site of pCRXI. The pCRXI vector contains the 6HisRa12 portion of the fusion. The Ra12 portion of the fusion construct encodes a polypeptide corresponding to amino acid residues 192-323 of Mycobacterium tuberculosis MTB32A, as described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference. The correct orientation of each insert was determined by its restriction enzyme pattern and its sequence was verified. Multiple fusion constructs were made for PmpA, PmpB, PmpC, PmpF and PmpH, as described further below:

# PMPA FUSION PROTEINS

PmpA is 107 kD protein containing 982 aa and was cloned from serovar

E. The PmpA protein was divided into 2 overlapping fragments, the PmpA(Nterminal) and (C-terminal) portions.

PmpA(N-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGTTTATAACAAAGGAACTTATG (SEQ ID NO:306)

GAGAGCGGCCGCTTACTTAGGTGAGAAGAAGGGAGTTTC (SEQ ID NO:307)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 308, encoding a 66 kD protein (619aa) expressing the segment 1-473 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 309.

PmpA(C-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCCATTCTATTCATTTCTTTGATCCTG (SEQ ID NO:310)

GAGAGCGCCCCTTAGAAGCCAACATAGCCTCC (SEQ ID NO:311)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 312, encoding a 74 kD protein (691aa) expressing the segment 438-982 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 313.

#### PMPF FUSION PROTEINS

PmpF is 112 kD protein containing 1034 aa and was cloned from the 25 serovar E. PmpF protein was divided into 2 overlapping fragments, the PmpF(N- term) and (C-term) portions.

PmpF(N-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGATTAAAAGAACTTCTCTATCC (SEQ ID NO:314)

30 GAGAGCGGCCGCTTATAATTCTGCATCATCTTCTATGGC (SEQ ID NO:315)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 316, encoding a 69 kD protein (646aa) expressing the segment 1-499 aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 317.

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PmpF(C-term) was amplified by the sense and antisense primers:

GAGAGCGCCCCTCGACATACGAACTCTGATGGG (SEQ ID NO:318)

GAGAGCGCCCCTTAAAAGACCAGAGCTCCTCC (SEQ ID NO:319)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 320, encoding a 77 kD protein (715aa) expressing the segment 466-1034aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 321.

# 10 PMPH FUSION PROTEINS

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PmpH is 108 kD protein containing 1016 as and was cloned from the serovar E. PmpH protein was divided into 2 overlapping fragments, the PmpH(N-term)and (C-term)portions.

PmpH(N-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCATGCCTTTTTCTTTGAGATCTAC (SEQ ID NO:322)

GAGAGCGCCGCTTACACAGATCCATTACCGGACTG (SEQ ID NO:323)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 324, 20 encoding a 64 kD protein (631aa) expressing the segment 1-484 aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 325.

PmpH(C-term) was amplified by the sense and antisense primers:

GAGAGCGCCGCTCGATCCTGTAGTACAAAATAATTCAGC (SEO ID NO:326)

25 GAGAGCGGCCGCTTAAAAGATTCTATTCAAGCC (SEQ ID NO:327)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 328, encoding a 77 kD protein (715aa) expressing the segment 449-1016aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 329.

PMPR FUSION PROTEINS

PmpB is 183 kD protein containing 1750 aa and was cloned from the serovar E. PmpB protein was divided into 4 overlapping fragments, PmpB(1), (2), (3) and (4).

35 PmpB(1) was amplified by the sense and antisense primers:

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GAGAGCGGCCGCTCATGAAATGGCTGTCAGCTACTGCG (SEQ ID NO:330)

GAGAGCGCCGCTTACTTAATGCGAATTTCTTCAAG (SEQ ID NO:331)

5 respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 332, and encodes is a 53 kD protein (518aa) expressing the segment 1-372 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 333.

PmpB(2) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGGTGACCTCTCAATTCAATCTTC (SEQ ID NO:334)

GAGAGCGGCCGCTTAGTTCTCTGTTACAGATAAGGAGAC (SEQ ID NO:335)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 336 and encodes a 60 kD protein (585aa) expressing the segment 330-767 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 337.

PmpB(3) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGACCAACTGAATATCTCTGAGAAC (SEQ ID NO:338)

GAGCGCCGCTTAAGAGACTACGTGGAGTTCTG (SEQ ID NO:339)

20

25

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 340 encodes a 67 kD protein (654aa) expressing the segment 732-1236 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 341

PmpB(4) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGGAACTATTGTGTTCTCTTCTG (SEQ ID NO:342)

GAGAGCGCCGCTTAGAAGATCATGCGAGCACCGC (SEQ ID NO:343)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID 30 NO: 344 encodes a 76 kD protein (700aa) expressing the segment 1160-1750 of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 345.

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## PMPC FUSION PROTEINS

PmpC is 187 kD protein containing 1774 aa and was cloned from the serovar E/L2. PmpC protein was divided into 3 overlapping fragments, PmpC(1), (2) and (3).

PmpC(1) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCATGAAATTTATGTCAGCTACTGC (SEQ ID NO:346)

GAGAGCGGCCGCTTACCCTGTAATTCCAGTGATGGTC (SEQ ID NO:347)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 348 and encodes a 51 kD protein (487aa) expressing the segment 1-340 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 349.

PmpC(2) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCGATACACAAGTATCAGAATCACC (SEQ ID NO:350)

GAGAGCGCCCCTTAAGAGGACGATGAGACACTCTCG (SEQ ID NO:351)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 352 and encodes a 60 kD protein (583aa) expressing the segment 305-741 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 353.

PmpC(3) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGATCAATCTAACGAAAACACAGACG (SEQ ID NO:354)

25 GAGAGCGGCCGCTTAGACCAAAGCTCCATCAGCAAC (SEQ ID NO:355)

30

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 356 and encodes a 70 kD protein (683aa) expressing the segment 714-1250 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 357.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

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#### CLAIMS

- 1. An isolated polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 175-180, 189-196, 264 and 266.
- An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
- 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
  - A host cell transformed with an expression vector according to claim 4.
- 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of E. coli, yeast and mammalian cells.
- 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
- 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.
- 9. A fusion protein according to claim 7, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.
- A fusion protein according to claim 7, wherein the fusion protein comprises an affinity tag.

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- An isolated polynucleotide encoding a fusion protein according to claim 7.
- 12. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.
- 13. A pharmaceutical composition comprising a polypeptide according to claim 1, and a physiologically acceptable carrier.
- 14. A pharmaceutical composition comprising a polynucleotide molecule according to claim 3 and a physiologically acceptable carrier.
- 15. A pharmaceutical composition comprising a polypeptide and a physiologically acceptable carrier, wherein the polypeptide is encoded by polynucleotide molecule selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 16. A pharmaceutical composition comprising a polynucleotide molecule and a physiologically acceptable carrier, wherein the polynucleotide molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 17. A pharmaceutical composition comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:
  - (a) a fusion protein according to claim 7;
  - (b) a polynucleotide according to claim 11; and
  - (c) an antibody according to claim 12.
- A vaccine comprising a polypeptide according to claim 1, and an immunostimulant.

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  19. A vaccine comprising a polynucleotide molecule according to claim 3 and an immunostimulant.
- 20. A vaccine comprising a polypeptide and an immunostimulant, wherein the polypeptide is encoded by a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 21. A vaccine comprising a DNA molecule and an immunostimulant, wherein the DNA molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 22. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:
  - (a) a fusion protein according to claim 7;
  - (b) a polynucleotide according to claim 11; and
  - (c) an antibody according to claim 12.
- 23. The vaccine of any one of claims 18-22 wherein the immunostimulant is an adjuvant.
- 24. A method for inducing protective immunity in a patient, comprising administering to a patient a pharmaceutical composition according to any one of claims 13-17.
- 25. A method for inducing protective immunity in a patient, comprising administering to a patient a vaccine according to any one of claims 18-22.
- 26. An isolated polyclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.

- 27. A method for detecting Chlamydia infection in a patient, comprising:
- (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291. (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (c) detecting the presence of antibodies that bind to the polypeptide.
  - 28. A method for detecting Chlamydia infection in a patient, comprising:
  - (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (c) detecting the presence of antibodies that bind to the fusion protein.
- 29. The method of any one of claims 27 and 28 wherein the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.
- 30. A method for detecting Chlamydia infection in a biological sample, comprising:
- (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the oligonucleotide primers is specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
- (b) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting Chlamydia infection.

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- 31. The method of claim 30, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide sequence of SEO ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 32. A method for detecting Chlamydia infection in a biological sample, comprising:
- (a) contacting the sample with one or more oligonucleotide probes specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
- (b) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe, thereby detecting *Chlamydia* infection.
- 33. The method of claim 32 wherein the probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291
- A method for detecting Chlamydia infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting Chlamydia infection in the biological sample.
- 35. A method of detecting Chlamydia infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEO ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences

complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and

- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting Chlamydia infection in the biological sample.
- 36. The method of any one of claims 34 and 35 wherein the binding agent is a monoclonal antibody.
- The method of any one of claims 34 and 35 wherein the binding agent is a polyclonal antibody.
- 38. The method of any one of claims 34 and 35 wherein the biological sample is selected from the group consisting of whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.
  - A diagnostic kit comprising:
- (a) a polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (b) a detection reagent.
  - 40. A diagnostic kit comprising:
- (a) a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
  - (b) a detection reagent.
- The kit of claims 39 or 40 wherein the polypeptide is immobilized on a solid support.

- 42. The kit of claims 39 or 40 wherein the detection reagent comprises a reporter group conjugated to a binding agent.
- 43. The kit of claim 42 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.
- 44. The kit of claim 42 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dve particles.
- 45. A diagnostic kit comprising at least two oligonucleotide primers, at least one of the oligonucleotide primers being specific for a polynucleotide molecule comprising a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 46. A diagnostic kit according to claim 43, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a sequence of SEO ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 47. A diagnostic kit comprising at least one oligonucleotide probe, the oligonucleotide probe being specific for a polynucleotide molecule comprising a sequence of SEO ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 48. A kit according to claim 47, wherein the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
  - 49. A diagnostic kit comprising:
- (a) at least one antibody, or antigen-binding fragment thereof, according to claim 22; and
  - (b) a detection reagent.
- 50. A method for treating Chlamydia infection in a patient, comprising the steps of:
  - (a) obtaining peripheral blood cells from the patient;

- (b) incubating the cells in the presence of at least one polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
  - (c) administering to the patient the proliferated T cells.
- 51. A method for treating Chlamydia infection in a patient, comprising the steps of:
  - (a) obtaining peripheral blood cells from the patient;
- (b) incubating the cells in the presence of at least one polynucleotide, comprises a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
  - (c) administering to the patient the proliferated T cells.
- 52. The method of any one of claims 50 and 51 wherein the step of incubating the T cells is repeated one or more times.
- 53. The method of any one of claims 50 and 51 wherein step (a) further comprises separating T cells from the peripheral blood cells, and the cells incubated in step (b) are the T cells.
- 54. The method of any one of claims 50 and 51 wherein step (a) further comprises separating CD4+ cells or CD8+ T cells from the peripheral blood cells, and the cells proliferated in step (b) are CD4+ or CD8+ T cells.
- 55. The method of any one of claims 50 and 51 wherein step (a) further comprises separating gamma/delta T lymphocytes from the peripheral blood cells, and the cells proliferated in step (b) are gamma/delta T lymphocytes.
- 56. The method of any one of claims 50 and 51 wherein step (b) further comprises cloning one or more T cells that proliferated in the presence of the polypeptide.

- 57. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polypeptide of claim 1. in combination with a physiologically acceptable carrier.
- 58. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 59. A method for treating Chlamydia infection in a patient, comprising the steps of:
- (a) incubating antigen presenting cells in the presence of at least one polypeptide of claim 1;
  - (b) administering to the patient the incubated antigen presenting cells.
- 60. A method for treating Chlamydia infection in a patient, comprising the steps of:
- (a) introducing at least one polynucleotide of claim 3 into antigen presenting cells;
  - (b) administering to the patient the antigen presenting cells.
- 61. The method of claims 59 or 60 wherein the antigen presenting cells are selected from the group consisting of dendritic cells. macrophage cells, B cells fibroblast cells, monocyte cells, and stem cells.
- 62. A pharmaceutical composition for the treatment of Chlamydia infection in a patient, comprising antigen presenting cells incubated in the presence of a polypeptide of claim I, in combination with a physiologically acceptable carrier.
- 63. A pharmaceutical composition for the treatment if Chlamydia infection in a patient, comprising antigen presenting cells incubated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 64. A polypeptide comprising an immunogenic portion of a Chlamydia antigen, wherein said immunogenic portion comprises a sequence of SEQ ID NO: 246, 247 and 254-256.

- 65. An immunogenic epitope of a *Chlamydia* antigen, comprising a sequence of SEQ ID NO: 246, 247 or 254-256.
- An isolated polypeptide comprising a sequence recited in any one of SEO ID NO: 224-262, 246, 247, 254-256, 292 and 294-305.
- . 67. A recombinant fusion polypeptide comprising a an amino acid sequence of a Ral2 polypeptide and an amino acid sequence of a Chlamydial polypeptide.
- 68. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a Pmp polypeptide.
- 69. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a PmpA, PmpF, PmpH, PmpB, or PmpC.
- 70. The recombinant polypeptide of claims 67, wherein the amino acid sequence of the fusion polypeptide is a sequence selected from the group consisting of SEQ ID NOs: 309, 313, 317, 321, 325, 329, 333, 337, 341, 345, 349, 353 and 357.
- 71. A recombinant DNA molecule encoding a fusion polypeptide according to claim 67.

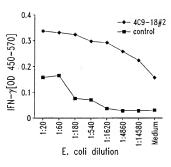
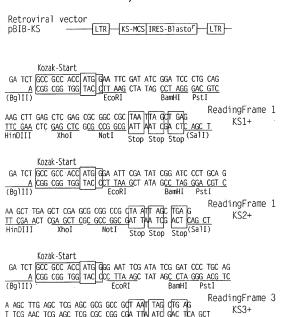


Fig. 1

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NotI

XhoI

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Chlamydia C17.8 Peptide Screen

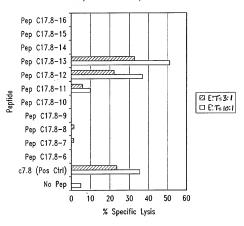
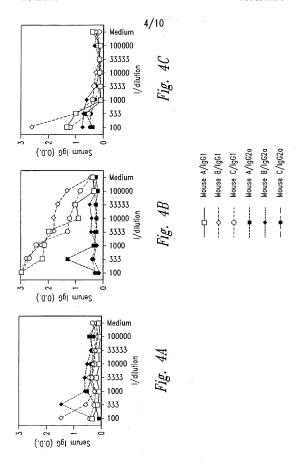
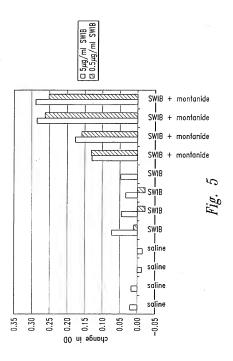


Fig. 3

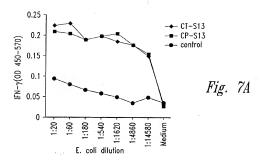


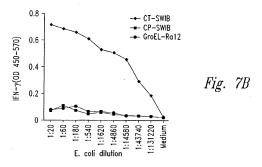
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Fig. 6





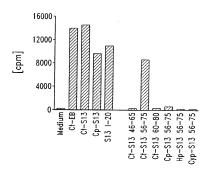
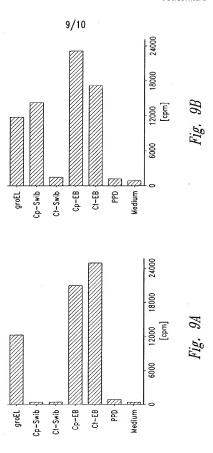


Fig. 8





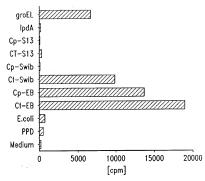


Fig. 10

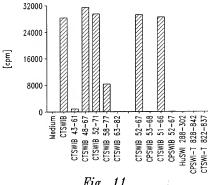


Fig. 11

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1

### SEQUENCE LISTING <110> Corixa Corporation Probst, Peter Bhatia, Ajay Skeiky, Yasir A. W. Fling, Steven P. Scholler, John <120> COMPOSITIONS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION <130> 210121 46901PC <140> PCT <141> 2000-12-04 <160> 357 <170> FastSEQ for Windows Version 3.0/4.0 <210> 1 <211> 481 <212> DNA <213> Chlamydia trachomatis <400> 1 ctgaagactt ggctatgttt tttattttga cgataaacct agttaaggca taaaagagtt 60 gcgaaggaag agccctcaac ttttcttatc accttcttta actaggagtc atccatgagt 120 caaaataaga actotgottt catgoagoot gtgaacgtat cogotgattt agotgocato 180 gttggtgcag gacctatgcc tcgcacagag atcattaaga aaatgtggga ttacattaag gagaatagtc ttcaagatcc tacaaacaaa cgtaatatca atcccgatga taaattggct 240 300 asagtttttg gaactgaaaa acctatcgat atgttccaaa tgacaaaaat ggtttctcaa 360 cacatcatta aataaaatag aaattgactc acgtgttcct cgtctttaag atgaqqaact 420 agttcattct ttttgttcgt ttttgtgggt attactgtat ctttaacaac tatcttagca 480 481 <210> 2 <211> 183 <212> DNA <213> Chlamydia trachomatis <400> 2 atcgttggtg caggacctat gcctcgcaca gagatcatta agaaaatgtg ggattacatt 60 aaggagaata gtottoaaga tootacaaac aaacgtaata toaatoooga tgataaattg 120 getaaagttt ttggaactga aaaacctate gatatgttee aaatgacaaa aatggtttet 180 183 <210> 3 <211> 110

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Tyr Le

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Arg Ala Ile Gly Lys Ala Val Ala Met Gly Glu Ala Asp Gly Phe Ala

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Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp

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Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 120

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PCT/US00/32919

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780 840 897

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Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lvs Ala Ala Ser Gln 105 Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser 120 125 His Lys Arg Arg Ala Ala Ala Val Cys Gly Phe Ile Gly Gly Ile 135 140 Thr Tyr Leu Ala Thr Phe Gly Val Ile Arg Pro Ile Leu Phe Val Asn 150 155 Lys Met Leu Val Asn Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met 165 170 175 Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val 180 185 190 Val Gly Ala Gly Leu Ala Ile Ser Ala Glu Arg Ala Asp Cys Glu Ala 200 205 Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Ser Gly 210 215 220 Glu Glu Asn Ala Cys Glu Lys Arg Val Ala Gly Glu Lys Ala Lys Thr 230 235 Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu 245 250 Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met 260 265 270 Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile 275 280 285 Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala 290 295

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ceacagetag atetitigig tjetcataaag egeaagagg etgeggets tetjaggete 420

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540

600

660

720

780

840

897

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Gln Arg Thr Ser Asp Gln Gly Leu Val Arg Asn Ala Ile Tyr Leu Xaa Lys Asp Ala Ile Leu Ser Ser Leu Glu Ala Arg Asp Gly Asp Ile Leu Phe Phe Asp Pro Ile Val Gln Glu Ser Ser Ser Lys Glu Ser Pro Leu Pro Ser Ser Leu Gln Ala Ser Val Thr Ser Pro Thr Pro Ala Thr Ala Ser Pro Leu Val Ile Gln Thr Ser Ala Asn Arg Ser Val Ile Phe Ser Ser Glu Arg Leu Ser Glu Glu Glu Lys Thr Pro Asp Asn Leu Thr Ser Gln Leu Gln Gln Pro Ile Glu Leu Lys Ser Gly Arg Leu Val Leu Lys Asp Arg Ala Val Leu Ser Ala Pro Ser Leu Ser Gln Asp Pro Gln Ala Leu Leu Ile Met Glu Ala Gly Thr Ser Leu Lys Thr Ser Ser Asp Leu Lys Leu Ala Thr Leu Ser Ile Pro Leu His Ser Leu Asp Thr Glu Lys Ser Val Thr Ile His Ala Pro Asn Leu Ser Ile Gln Lys Ile Phe Leu Ser Asn Ser Gly Asp Glu Asn Phe Tyr Glu Asn Val Glu Leu Leu Ser Lys Glu Gln Asn Asn Ile Pro Leu Leu Thr Leu Pro Lys Glu Gln Ser His Leu His Leu Pro Asp Gly Asn Leu Ser Ser His Phe Gly Tyr Gln Gly Asp Trp Thr Phe Ser Trp Lys Asp Ser Asp Glu Gly His Ser Leu Ile Ala Asn Trp Thr Pro Lys Asn Tyr Val Pro His Pro Glu Arg Gln Ser Thr Leu Val Ala Asn Thr Leu Trp Asn Thr Tyr Ser Asp Met Gln Ala Val Gln Ser Met Ile Asn Thr Thr Ala His Gly Gly Ala Tyr Leu Phe Gly Thr Trp Gly Ser Ala Val Ser Asn Leu Phe Tyr Val His Asp Ser Ser Gly Lys Pro Ile Asp Asn Trp His His Arg Ser Leu Gly Tyr Leu Phe Gly Ile Ser Thr His Ser Leu Asp Asp His Ser Phe Cys Leu Ala Ala Gly Gln Leu Leu Gly Lys Ser Ser Asp Ser Phe Ile Thr Ser Thr Glu Thr Thr Ser Tyr Ile Ala Thr Val Gln Ala Gln Leu Ala Thr Ser Leu Met Lys Ile Ser Ala Gln Ala Cys Tyr Asn Glu Ser Ile His Glu Leu Lys Thr Lys Tyr Arg Ser Phe Ser Lys Glu Gly Phe Gly Ser Trp His Ser Val Ala Val Ser Gly Glu Val Cys Ala Ser Ile Pro Ile Val Ser Asn Gly Ser Gly Leu Phe Ser Ser Phe Ser Ile Phe Ser Lys Leu Gln Gly Phe Ser Gly Thr Gln Asp Gly Phe Glu Glu Ser Ser Gly 755 760 Glu Ile Arg Ser Phe Ser Ala Ser Ser Phe Arg Asn Ile Ser Leu Pro 

Ile Gly Ile Thr Phe Glu Lys Lys Ser Gln Lys Thr Arg Thr Tyr Tyr 790 795 Tyr Phe Leu Gly Ala Tyr Ile Gln Asp Leu Lys Arg Asp Val Glu Ser 805 810 Gly Pro Val Val Leu Leu Lys Asn Ala Val Ser Trp Asp Ala Pro Met 825 Ala Asn Leu Asp Ser Arg Ala Tyr Met Phe Arg Leu Thr Asn Gln Arg 840 Ala Leu His Arg Leu Gln Thr Leu Leu Asn Val Ser Cys Val Leu Arg 855 Gly Gln Ser His Ser Tyr Ser Leu Asp Leu Gly Thr Thr Tyr Arg Phe 870 875

<210> 176

<211> 982 <212> PRT

<213> Chlamydia

<220> <221> VARIANT

<222> (1)...(982) <223> Xaa = Anv Amino Acid

<400× 176 Met Ile Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val Ser Phe 1 10 Pro Tyr Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro 35 40 Leu Ser Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg 55 Gly His Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn Gly Ala 70 75 Ala Leu Ser Asn Ser Ala Ala Asp Gly Leu Phe Thr Ile Glu Gly Phe 85 90 Lys Glu Leu Ser Phe Ser Asn Cys Asn Ser Leu Leu Ala Val Leu Pro 105 Ala Ala Thr Thr Asn Lys Gly Ser Gln Thr Pro Thr Thr Thr Ser Thr 120 125 Pro Ser Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu Leu Asn 135 Asn Glu Lys Phe Ser Phe Tyr Ser Asn Leu Val Ser Gly Asp Gly Gly 150 155 Ala Ile Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys Leu Cys 165 170 Val Phe Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys Gln Val 185 Val Thr Ser Phe Ser Ala Met Ala Asn Glu Ala Pro Ile Ala Phe Val 200 Ala Asn Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val Gln Asp 215 220 Gly Gln Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro Val Val 230 235 Ser Phe Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val Ala Arg 245 250 Val Gly Gly Gly Ile Tyr Ser Tyr Gly Asn Val Ala Phe Leu Asn Asn 260 265

Gly	Lys	Thr 275	Leu	Phe	Leu	Asn	Asn 280		Ala	Ser	Pro	Val 285	Tyr	Ile	Ala
Ala	Lys 290	Gln	Pro	Thr	Ser	Gly 295			Ser	Asn	Thr 300	Ser	Asn	Asn	Туз
Gly 305	Asp	Gly	Gly	Ala	Ile 310	Phe	Cys	Lys	Asn	Gly 315	Ala	Gln	Ala	Gly	Ser 320
Asn	Asn	Ser	Gly	Ser 325	Val	Ser	Phe	Asp	Gly 330	Glu	Gly	Val	Val	Phe 335	Phe
Ser	Ser	Asn	Val 340	Ala	Ala	Gly	Lys	Gly 345		Ala	Ile	Tyr	Ala 350	Lys	Lys
Leu	Ser	Val 355	Ala	Asn	Cys	Gly	Pro 360	Val	Gln	Phe	Leu	Arg 365	Asn	Ile	Ala
Asn	Asp 370	Gly	Gly	Ala	Ile	Tyr 375	Leu	Gly	Glu	Ser	Gly 380	Glu	Leu	Ser	Leu
385			_	_	390					Gly 395			-		400
	-			405		_			410	Val				415	
			420					425		Thr			430		
		435					440			Ile		445			
	450					455				Leu	460				
465					470					Asn 475					480
				485					490	Ile				495	
			500					505		Thr			510		
		515	_				520			Thr		525			
	530					535				Leu Val	540				
545					550					555 Val					560
				565	_				570	Phe				575	
yan	_		580				-	585		Gly			590		_
Acp		595					600			Pro		605			
	610					615				Lys	620				
625					630					635 Ala					640
				645					650	Tyr			-	655	
			660					665		Trp			670	Leu	
		675					680			Ser		685			-
	690					695				Ser	700	-	-	_	
705					710					715 Tyr					720
usb	wrd	vab	wrg	725	GIĀ	GIII	GIĀ	-yr	730	.yr	TTE	ser	GIY	735	.yr

Ser Leu Gly Ala Asn Ser Tyr Phe Gly Ser Ser Met Phe Gly Leu Ala 740 745 Phe Thr Glu Val Phe Gly Arg Ser Lys Asp Tyr Val Val Cys Arg Ser 760 765 Asn His His Ala Cys Ile Gly Ser Val Tyr Leu Ser Thr Gln Gln Ala 775 780 Leu Cys Gly Ser Tyr Leu Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr 790 795 Gly Phe Gly Asn Gln His Met Lys Thr Ser Tyr Thr Phe Ala Glu Glu 805 810 Ser Asp Val Arg Trp Asp Asn Asn Cys Leu Ala Gly Glu Ile Gly Ala 825 Gly Leu Pro Ile Val Ile Thr Pro Ser Lys Leu Tyr Leu Asn Glu Leu 840 845 Arg Pro Phe Val Gln Ala Glu Phe Ser Tyr Ala Asp His Glu Ser Phe 855 860 Thr Glu Glu Gly Asp Gln Ala Arg Ala Phe Lys Ser Gly His Leu Leu 870 875 Asn Leu Ser Val Pro Val Gly Val Lys Phe Asp Arg Cys Ser Ser Thr 890 885 His Pro Asn Lys Tyr Ser Phe Met Ala Ala Tyr Ile Cys Asp Ala Tyr 900 905 Arg Thr Ile Ser Gly Thr Glu Thr Thr Leu Leu Ser His Gln Glu Thr 915 920 925 Trp Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Val Val Arg 935 940 Gly Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His 950 955 Gly Arg Tyr Glu Tyr Arg Asp Ala Ser Arg Gly Tyr Gly Leu Ser Ala 965 970 Gly Ser Lys Val Xaa Phe 980

<400> 177 Met Lys Lys Ala Phe Phe Phe Leu Ile Gly Asn Ser Leu Ser Gly 1 10 Leu Ala Arg Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val 20 25 Pro Asp Pro Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly 40 Asp Thr His Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile 55 Leu Ala Ile Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile 70 Thr Asp Tyr Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe Ala Lys Asn Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser 100 105 110 Pro Asn Ser Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile 120 125 Phe Glu Asn Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr 135 140 Ala Ala Asp Lys Ile Arg Glu Gly Gly Ala Ile His Ala Gln Asn Leu

<sup>&</sup>lt;210> 177

<sup>&</sup>lt;211> 964

<sup>&</sup>lt;212> PRT <213> Chlamydia

145					150					155					160
Tyr	Ile	Asn	His	Asn	His	Asp	Val	Val	Gly	Phe	Met	Lys	Asn	Phe	Ser
				165					170					175	
Tyr	Val	Gln	Gly	Gly	Ala	Ile	Ser	Thr	Ala	Asn	Thr	Phe	Val	Val	Ser
			180					185					190		
Glu	Asn	Gln		Cvs	Phe	Leu	Phe		Asp	Asn	Ile	Cvs	Ile	Gln	Thr
		195		-1-			200		E			205			
λen	Thr		Glv	Lvs	Glv	Glv		Tle	Tur	Δla	Glv		Ser	Δen	Ser
	210	1120		-7.0		215			-1-		220	****			
Dhe		Cor	7 en	7 en	Cve		Tou	Dho	Dho	T10		λen	Ala	Cva	Cva
225	GIU	ser	Moli	Aon	230	льр	пец	File	File	235	Maii	Mair	мта	Cys	240
	0111	01	212	T10		000	Dva	Tlo	Crea		T 011	mha	Gly	) an	
мта	Gry	Gry	ALG	245	Fire	ser	FIO	TTE	250	ser	ьец	1111	GLY	255	ALG
G1		T1 -	*** 7		m	n				n					m)
GIY	ABII	TTE	260	PHe	TAT	ASII	ASII	265	Cys	rne	гув	ASII	Val 270	GIU	TIIL
.1.							a1			-1.				m)	
ALA	ser		GIU	Ата	ser	Asp		GIY	Ala	тте	гÀа		Thr	Thr	Arg
_	_	275			_	_	280	_				285	_	_	
Leu		vai	Thr	GIY	Asn		GIY	Arg	шe	Pne		ser	Asp	Asn	IIe
	290	_	_			295					300	_			_
	Lys	Asn	Tyr	Gly		Ala	Ile	Tyr	Ala		Val	Val	Thr	Leu	
305					310					315					320
Asp	Asn	Gly	Pro		Tyr	Phe	Ile	Asn		Ile	Ala	Asn	Asn		Gly
	_		_	325					330			_		335	
Gly	Ala	Ile		Ile	Asp	Gly	Thr		Asn	Ser	Lys	Ile	Ser	Ala	Asp
		_	340					345					350		
Arg	His		Ile	Ile	Phe	Asn		Asn	Ile	val	Thr		Val	Thr	Asn
		355					360					365			
Ala		Gly	Thr	Ser	Thr		Ala	Asn	Pro	Pro		Arg	Asn	Ala	Ile
	370					375					380				
	Val	Ala	Ser	Ser		Gly	Glu	Ile	Leu		Gly	Ala	Gly	Ser	
385					390					395					400
Gln	Asn	Leu	Ile		Tyr	Asp	Pro	Ile		Val	Ser	Asn	Ala		Val
				405					410					415	
Ser	Val	Ser		Asn	Lys	Glu	Ala		Gln	Thr	Gly	Ser	Val	Val	Phe
			420					425					430		
Ser	Gly		Thr	Val	Asn	Ser		Asp	Phe	His	Gln		Asn	Leu	Gln
		435					440					445			
Thr	Lys	Thr	Pro	Ala	Pro	Leu	Thr	Leu	Ser	Asn	Gly	Phe	Leu	Cys	Ile
	450					455					460				
Glu	Asp	His	Ala	Gln	Leu	Thr	Val	Asn	Arg	Phe	Thr	Gln	Thr	Gly	Gly
465					470					475					480
Val	Val	Ser	Leu	Gly	Asn	Gly	Ala	Val	Leu	Ser	Cys	Tyr	Lys	Asn	Gly
				485					490					495	
Thr	Gly	Asp	Ser	Ala	Ser	Asn	Ala	Ser	Ile	Thr	Leu	Lys	His	Ile	Gly
			500					505					510		
Leu	Asn	Leu	Ser	Ser	Ile	Leu	Lys	Ser	Gly	Ala	Glu	Ile	Pro	Leu	Leu
		515					520					525			
Trp	Val	Glu	Pro	Thr	Asn	Asn	Ser	Asn	Asn	Tyr	Thr	Ala	Asp	Thr	Ala
	530					535				-	540		-		
Ala	Thr	Phe	Ser	Leu	Ser	Asp	Val	Lys	Leu	Ser	Leu	Ile	Asp	Asp	Tyr
545					550	-		•		555			-	•	560
Glv	Asn	Ser	Pro	Tvr	Glu	Ser	Thr	Asp	Leu	Thr	His	λla	Leu	Ser	Ser
,				565					570					575	
Glr	Pro	Met	Lev		Ile	Ser	Glυ	Ala		Asp	Asp	Glp	Leu		Ser
			580			501		585	DUL	p		J.11	590	J.11	
Gla	Agn	Tle		Dhe	Ser	Glv	Len		Va l	Dro	Hie	Tur	Gly	Trn	Glr
		595	p		DGI	y	600		•41			605	- Y		U_11
Glv	T.011		Thr	Trr	GIV	Trr		Lve	Thr	G1r	Ner		Glu	Dro	Δls
		~ + P	-111	** 5	OT A	~ + 1	*TT CI	ت ر ب	*111	CTIL	чэр		JIU	-10	**** cr

615 620 Ser Ser Ala Thr Ile Thr Asp Pro Gln Lys Ala Asn Arg Phe His Arg 630 635 Thr Leu Leu Leu Thr Trp Leu Pro Ala Gly Tyr Val Pro Ser Pro Lys 645 650 His Arg Ser Pro Leu Ile Ala Asn Thr Leu Trp Gly Asn Met Leu Leu 665 670 Ala Thr Glu Ser Leu Lys Asn Ser Ala Glu Leu Thr Pro Ser Gly His 675 680 Pro Phe Trp Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln 695 Asp Pro Arg Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr 710 715 Ser Ala Gly Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe 725 730 Ser Gln Thr Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val 745 740 Ser Ser Lys Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln 760 Glu Gly Phe Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp 770 775 His Asn Cys His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln 790 795 Gly Thr Phe Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu 805 810 815 Pro Met Lys Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu 825 830 Gly Ala Leu Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly 840 845 Ala Tyr Pro Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu 850 855 860 Val Pro Ile Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro 870 875 Gln Ala Trp Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln 890 885 Glu Pro Gly Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe 905 Gly Ser Gly Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser 920 925 915 Gln Gln Thr Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His 935 Gly Phe Tyr Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile 955 945 950 Ala Leu Arg Phe

<210> 178 <211> 1530 <212> PRT

<213> Chlamvdia

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Gly Pro Gln Ala Val Leu Leu Leu Asp Gln Ile Arg Asp Leu Phe Val 55 Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly 75 70 Asp Pro Ser Ser Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys 8.5 90 Val Glu Gln Ser Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln 100 105 Gly Val Asp Gln Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser 120 Phe Thr Ser Ser Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu 135 Gly Ile Ala Phe Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr 150 155 Asp Val Lys Ala Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp 165 170 Leu Ile Phe Glu Lys Ile Lys Gly Gly Leu Glu Phe Ala Ser Cys Ser 185 Ser Leu Glu Gln Gly Gly Ala Cys Ala Ala Gln Ser Ile Leu Ile His 200 Asp Cys Gln Gly Leu Gln Val Lys His Cys Thr Thr Ala Val Asn Ala 215 Glu Gly Ser Ser Ala Asn Asp His Leu Gly Phe Gly Gly Gly Ala Phe 230 235 Phe Val Thr Gly Ser Leu Ser Gly Glu Lys Ser Leu Tyr Met Pro Ala 245 250 Gly Asp Met Val Val Ala Asn Cys Asp Gly Ala Ile Ser Phe Glu Gly 265 Asn Ser Ala Asn Phe Ala Asn Gly Gly Ala Ile Ala Ala Ser Gly Lys 280 Val Leu Phe Val Ala Asn Asp Lys Lys Thr Ser Phe Ile Glu Asn Arg 295 Ala Leu Ser Gly Gly Ala Ile Ala Ala Ser Ser Asp Ile Ala Phe Gln 310 315 Asn Cys Ala Glu Leu Val Phe Lys Gly Asn Cys Ala Ile Gly Thr Glu 325 330 Asp Lys Gly Ser Leu Gly Gly Gly Ala Ile Ser Ser Leu Gly Thr Val 345 Leu Leu Gln Gly Asn His Gly Ile Thr Cys Asp Lys Asn Glu Ser Ala 360 Ser Gln Gly Gly Ala Ile Phe Gly Lys Asn Cys Gln Ile Ser Asp Asn 375 380 Glu Gly Pro Val Val Phe Arg Asp Ser Thr Ala Cys Leu Gly Gly Gly 390 395 Ala Ile Ala Ala Gln Glu Ile Val Ser Ile Gln Asn Asn Gln Ala Gly 405 410 Ile Ser Phe Glu Gly Gly Lys Ala Ser Phe Gly Gly Gly Ile Ala Cys 420 425 Gly Ser Phe Ser Ser Ala Gly Gly Ala Ser Val Leu Gly Thr Ile Asp 440 445 Ile Ser Lys Asn Leu Gly Ala Ile Ser Phe Ser Arg Thr Leu Cys Thr 455 Thr Ser Asp Leu Gly Gln Met Glu Tyr Gln Gly Gly Ala Leu Phe 470 475 Gly Glu Asn Ile Ser Leu Ser Glu Asn Ala Gly Val Leu Thr Phe Lys 490 Asp Asn Ile Val Lys Thr Phe Ala Ser Asn Gly Lys Ile Leu Gly Gly 500 505

Gly Ala Ile Leu Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly 520 Gly Ile Ser Phe Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr 535 Gln Glu Glu Phe Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser 550 555 Ser Gly Tyr Ser Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile 565 570 Leu His Asn Ala Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser 585 Glu Glu Glu Ala Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His 600 Gly Met Asp Ser Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly 615 620 Asn Asn Tyr Ala Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser 630 635 Lys Thr Val Gln Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn 645 650 Ile Ala Ser Leu Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys 665 670 Glu Leu Val Asp Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg 680 Val Tyr Gly Gly Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser 690 695 700 Gly Asn Lys Gly Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu 710 715 Tyr Val Glu Glu Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro 725 730 Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln 745 Ser Phe Ile Thr Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly 760 Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg 775 Arg Glu Cys Ala Gly Gly Ala Ile Phe Ala Lys Arg Val Arg Ile Val 790 795 Asp Asn Gln Glu Ala Val Val Phe Ser Asn Asn Phe Ser Asp Ile Tyr 805 810 Gly Gly Ala Ile Phe Thr Gly Ser Leu Arg Glu Glu Asp Lys Leu Asp 820 825 Gly Gln Ile Pro Glu Val Leu Ile Ser Gly Asn Ala Gly Asp Val Val 835 840 Phe Ser Gly Asn Ser Ser Lys Arg Asp Glu His Leu Pro His Thr Gly 855 Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr Ile Ser Gln Asn Thr Gly 870 Asn Val Leu Phe Tyr Asn Asn Val Ala Cys Ser Gly Gly Ala Val Arg 890 Ile Glu Asp His Gly Asp Val Leu Leu Glu Ala Phe Gly Gly Asp Ile 905 Val Phe Lys Gly Asn Ser Ser Phe Arg Ala Gln Gly Ser Asp Ala Ile 915 920 Tyr Phe Ala Gly Lys Glu Ser His Ile Thr Ala Leu Asn Ala Thr Glu 935 940 Gly His Ala Ile Val Phe His Asp Ala Leu Val Phe Glu Asn Leu Lys 950 955 960 Glu Arg Lys Ser Ala Glu Val Leu Leu Ile Asn Ser Arg Glu Asn Pro 965 970

Gly Tyr Thr Gly Ser Ile Arg Phe Leu Glu Ala Glu Ser Lys Val Pro 985 Gln Cys Ile His Val Gln Gln Gly Ser Leu Glu Leu Leu Asn Gly Ala 1000 Thr Leu Cys Ser Tyr Gly Phe Lys Gln Asp Ala Gly Ala Lys Leu Val 1015 1020 Leu Ala Ala Gly Ser Lys Leu Lys Ile Leu Asp Ser Gly Thr Pro Val 1030 1035 1040 Gln Gly His Ala Ile Ser Lys Pro Glu Ala Glu Ile Glu Ser Ser Ser 1045 1050 1055 Glu Pro Glu Gly Ala His Ser Leu Trp Ile Ala Lys Asn Ala Gln Thr 1060 1065 1070 Thr Val Pro Met Val Asp Ile His Thr Ile Ser Val Asp Leu Ala Ser 1080 1085 Phe Ser Ser Ser Gln Gln Glu Gly Thr Val Glu Ala Pro Gln Val Ile 1095 1100 Val Pro Gly Gly Ser Tyr Val Arg Ser Gly Glu Leu Asn Leu Glu Leu 1110 1115 Val Asn Thr Thr Gly Thr Gly Tyr Glu Asn His Ala Leu Leu Lys Asn 1125 1130 1135 Glu Ala Lys Val Pro Leu Met Ser Phe Val Ala Ser Ser Asp Glu Ala 1140 1145 1150 Ser Ala Glu Ile Ser Asn Leu Ser Val Ser Asp Leu Gln Ile His Val 1155 1160 1165 Ala Thr Pro Glu Ile Glu Glu Asp Thr Tyr Gly His Met Gly Asp Trp 1175 1180 Ser Glu Ala Lys Ile Gln Asp Gly Thr Leu Val Ile Asn Trp Asn Pro 1190 1195 1200 Thr Gly Tyr Arg Leu Asp Pro Gln Lys Ala Gly Ala Leu Val Phe Asn 1205 1210 Ala Leu Trp Glu Glu Gly Ala Val Leu Ser Ala Leu Lys Asn Ala Arg 1225 Phe Ala His Asn Leu Thr Ala Gln Arg Met Glu Phe Asp Tyr Ser Thr 1235 1240 1245 Asn Val Trp Gly Phe Ala Phe Gly Gly Phe Arg Thr Leu Ser Ala Glu 1255 1260 Asn Leu Val Ala Ile Asp Gly Tyr Lys Gly Ala Tyr Gly Gly Ala Ser 1270 1275 Ala Gly Val Asp Ile Gln Leu Met Glu Asp Phe Val Leu Gly Val Ser 1285 1290 Gly Ala Ala Phe Leu Gly Lys Met Asp Ser Gln Lys Phe Asp Ala Glu 1305 1310 Val Ser Arg Lys Gly Val Val Gly Ser Val Tyr Thr Gly Phe Leu Ala 1320 1325 Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser Leu Gly Glu Thr Gln Asn 1330 1335 1340 Asp Met Lys Thr Arg Tyr Gly Val Leu Gly Glu Ser Ser Ala Ser Trp 1350 1355 Thr Ser Arg Gly Val Leu Ala Asp Ala Leu Val Glu Tyr Arg Ser Leu 1365 1370 1375 Val Gly Pro Val Arg Pro Thr Phe Tyr Ala Leu His Phe Asn Pro Tyr 1380 1385 1390 Val Glu Val Ser Tyr Ala Ser Met Lys Phe Pro Gly Phe Thr Glu Gln 1395 1400 1405 Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala Ser Leu Thr Asn Ile Thr 1410 1415 1420 Ile Pro Leu Gly Met Lys Phe Glu Leu Ala Phe Ile Lys Gly Gln Phe 1430 1435

PCT/US00/32919 75

Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr Ala Trp Glu Ala Tyr Arg 1445 1450 Lvs Val Glu Glv Glv Ala Val Gln Leu Leu Glu Ala Glv Phe Asp Trp 1465 Glu Gly Ala Pro Met Asp Leu Pro Arg Gln Glu Leu Arg Val Ala Leu 1475 1480 1485 Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu 1495 1500 Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr 1505 1510 1515 Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 1525 1530

<210> 179

<211> 1776 <212> PRT

<213> Chlamydia

1 5

<400> 179

Ser Ser Val Thr Glu Ala Ser Ser Ile Gln Asp Gln Ile Lys Asn Thr 20 25 3.0 Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln Ala Phe Thr 40 45 Asp Met Met Leu Ala Asp Asn Thr Glu Tyr Arg Ala Ala Asp Ser Val 55 60 Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arg Lys His Leu 70 . 75 Ser Ser Ser Ser Glu Ala Ser Pro. Thr Thr Glu Gly Val Ser Ser Ser 85 90 Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala Pro Ser Ser

Ala Ile Met Lys Phe Met Ser Ala Thr Ala Val Phe Ala Ala Val Leu

10

15

175

100 105 Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn Gly Gly Ile 120 Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln Asp Ser Leu

135 Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe Phe Gly Glu 150 155 160 Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn Gly Gly Ala

170 Ile Tyr Gly Glu Lys Glu Val Val Phe Glu Asn Ile Lys Ser Leu Leu 180 185 Val Glu Val Asn Ile Ser Val Glu Lys Gly Gly Ser Val Tyr Ala Lys

165

195 200 Glu Arq Val Ser Leu Glu Asn Val Thr Glu Ala Thr Phe Ser Ser Asn 215

Gly Gly Glu Gln Gly Gly Gly Ile Tyr Ser Glu Gln Asp Met Leu 230 235 Ile Ser Asp Cys Asn Asn Val His Phe Gln Gly Asn Ala Ala Gly Ala 245 250

Thr Ala Val Lys Gln Cys Leu Asp Glu Glu Met Ile Val Leu Leu Thr 260 265 270 Glu Cys Val Asp Ser Leu Ser Glu Asp Thr Leu Asp Ser Thr Pro Glu

275 280 285 Thr Glu Gln Thr Lys Ser Asn Gly Asn Gln Asp Gly Ser Ser Glu Thr 295 300

Lys Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser Pro Asp

305					310					315					320
Asp	Val	Leu	Gly	Lys 325	Gly	Gly	Gly	Ile	Tyr 330		Glu	Lys	Ser	Leu 335	
Ile	Thr	Gly	Ile 340	Thr	Gly	Thr	Ile	Asp 345	Phe	Val	Ser	Asn	Ile 350	Ala	Thr
Asp	Ser	Gly 355		Gly	Val	Phe	Thr 360	Lys	Glu	Asn	Leu	Ser 365		Thr	Asn
Thr	Asn 370		Leu	Gln	Phe	Leu 375			Ser	Ala	Gly 380		His	Gly	Gly
Gly 385		Tyr	Val	Thr	Gln 390		Met	Ser	Val	Thr 395		Thr	Thr	Ser	Glu 400
	Ile	Thr	Thr	Pro		Leu	Val	Gly	Glu 410		Ile	Phe	Ser	Glu 415	
Thr	Ala	Lys	Gly 420	His	Gly	Gly	Gly	Ile 425		Thr	Asn	Lys	Leu 430		Leu
Ser	Asn	Leu 435		Thr	Val	Thr	Leu 440		Lys	Asn	Ser	Ala 445		Glu	Ser
Gly	Gly 450		Ile	Phe	Thr	Asp		Ala	Ser	Ile	Pro		Thr	Asp	Thr
Pro 465	Glu	Ser	Ser	Thr	Pro 470	Ser	Ser	Ser	Ser	Pro 475	Ala	Ser	Thr	Pro	Glu 480
Val	Val	Ala	Ser	Ala 485	Lys	Ile	Asn	Arg	Phe 490	Phe	Ala	Ser	Thr	Ala 495	Glu
Pro	Ala	Ala	Pro 500	Ser	Leu	Thr	Glu	Ala 505	Glu	Ser	Asp	Gln	Thr 510	Asp	Gln
Thr	Glu	Thr 515	Ser	Asp	Thr	Asn	Ser 520	Asp	Ile	Asp	Val	Ser 525	Ile	Glu	Asn
Ile	Leu 530	Asn	Val	Дlа	Ile	Asn 535	Gln	Asn	Thr	Ser	Ala 540	Lys	Lys	Gly	Gly
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				Ser 565			_		570		_		_	575	
			580	Phe	_			585					590	-	
		595	-	Glu	-	-	600				-	605			
	610			Ser		615				_	620				
625				Thr	630					635					640
-				Thr 645					650					655	-
			660	Thr				665					670		
	_	675	-	Val			680				-	685		-	
	690			Ser		695					700				
705				Leu	710					715					720
				Ser 725					730					735	
			740	Ser	-		-	745					750	-	-
		75 <b>5</b>		Lys			760					765			
. r . ct	cys	ne a	urq	-ya	Ser	~ Y -	• • • • d	• s r d	2er	TIIL	Heb	Ser	Ser	2.10	val

775 780 Ser Asn Ser Ser Gly Ser Asp Val Thr Ala Ser Ser Asp Asn Pro Asp 790 795 Ser Ser Ser Ser Gly Asp Ser Ala Gly Asp Ser Glu Gly Pro Thr Glu 805 810 815 Pro Glu Ala Gly Ser Thr Thr Glu Thr Pro Thr Leu Ile Gly Gly 820 825 Ala Ile Tyr Gly Glu Thr Val Lys Ile Glu Asn Phe Ser Gly Gln Gly 835 840 845 Ile Phe Ser Gly Asn Lys Ala Ile Asp Asn Thr Thr Glu Gly Ser Ser 855 Ser Lys Ser Asn Val Leu Gly Gly Ala Val Tyr Ala Lys Thr Leu Phe 870 875 Asn Leu Asp Ser Gly Ser Ser Arg Arg Thr Val Thr Phe Ser Gly Asn 885 890 Thr Val Ser Ser Gln Ser Thr Thr Gly Gln Val Ala Gly Gly Ala Ile 905 910 Tyr Ser Pro Thr Val Thr Ile Ala Thr Pro Val Val Phe Ser Lys Asn 920 925 Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr Gln Arg Lys Asp 935 940 Thr Phe Gly Gly Ala Ile Gly Ala Thr Ser Ala Val Ser Leu Ser Gly 950 955 Gly Ala His Phe Leu Glu Asn Val Ala Asp Leu Gly Ser Ala Ile Gly 965 970 Leu Val Pro Asp Thr Gln Asn Thr Glu Thr Val Lys Leu Glu Ser Gly 980 985 Ser Tyr Tyr Phe Glu Lys Asn Lys Ala Leu Lys Arg Ala Thr Ile Tyr 995 1000 1005 Ala Pro Val Val Ser Ile Lys Ala Tyr Thr Ala Thr Phe Asn Gln Asn 1015 1020 Arg Ser Leu Glu Glu Gly Ser Ala Ile Tyr Phe Thr Lys Glu Ala Ser 1030 1035 Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn Leu Val Thr Pro 1045 1050 1055 Thr Leu Ser Thr Thr Thr Glu Gly Thr Pro Ala Thr Thr Ser Gly Asp 1065 1070 Val Thr Lys Tyr Gly Ala Ala Ile Phe Gly Gln Ile Ala Ser Ser Asn 1075 1080 1085 Gly Ser Gln Thr Asp Asn Leu Pro Leu Lys Leu Ile Ala Ser Gly Gly 1090 1095 1100 Asn Ile Cys Phe Arg Asn Asn Glu Tyr Arg Pro Thr Ser Ser Asp Thr 1105 1110 1115 1120 Gly Thr Ser Thr Phe Cys Ser Ile Ala Gly Asp Val Lys Leu Thr Met 1125 1130 1135 Gln Ala Ala Lys Gly Lys Thr Ile Ser Phe Phe Asp Ala Ile Arg Thr 1145 1150 Ser Thr Lys Lys Thr Gly Thr Gln Ala Thr Ala Tyr Asp Thr Leu Asp 1155 1160 1165 Ile Asn Lys Ser Glu Asp Ser Glu Thr Val Asn Ser Ala Phe Thr Gly 1175 1180 Thr Ile Leu Phe Ser Ser Glu Leu His Glu Asn Lys Ser Tyr Ile Pro 1195 1200 1190 Gln Asn Val Val Leu His Ser Gly Ser Leu Val Leu Lys Pro Asn Thr 1210 1215 1205 Glu Leu His Val Ile Ser Phe Glu Gln Lys Glu Gly Ser Ser Leu Val 1220 1225 Met Thr Pro Gly Ser Val Leu Ser Asn Gln Thr Val Ala Asp Gly Ala

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Gly Ile Ala 1265	Glu Gly	Asn Ile	Phe Tl	nr Pro	Pro Glu 1275	Leu	Arg Ile	Ile 1280
Asp Thr Thr	Thr Ser		Gly G	ly Thr 129	Pro Ser	Thr	Asp Ser	Glu
Ser Asn Gln								
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Thr Pro Thr	Thr Thr		Ala Ti	ır Thr	Thr Thr	Ser .	Asn Gln	Val
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Gln Asn Pro	1380		13	385			1390	
Pro Thr Asp 139	5		1400			1405		
Asp Ile Ala	Pro Gln			ır Gly			Leu Asp	Pro
1410		141			142			_
Asp Gln Leu 1425	GIN ASN	1430	IIe Se	er Ala	Leu Trp	Lys .	Pne Asp	Ser 1440
Tyr Arg Gln	Trn Ala		Dro A	or Ton		Dho !	Tree Ala	
Tyr Arg Gin	11p A1a		PIO AI	.9 ASD 1450		PHE	191 A14	
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Asn Leu Trp		Glv Leu		r Met	Leu Ser			Thr
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Leu Asp Ala			Asp Va			Ala A		
Lys Met Ile						Asn A	153: Asn Tyr	
	1540			45			1550	_
His Lys Gly	5		1560			1565		
Pro Phe His	Phe Val			s Thr			Leu Pro	Leu
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1585	GIY VAI	1590	Tyr GI		1595	nis A	asp Int	1600
Thr His Tyr	Pro Thr	Ile Arg	Glu Ar		Gln Gly	Glu 7	Trp Glu 161	Asp
Leu Gly Trp								
Ala Gln Gly	Asp Thr	Lys Arg	Ile Th		Tyr Gly	Glu I		Tyr
1639 Ser Ser Ile		Tara Gla	1640	r Cl.,	The Clu	1645	an Dro	7.20
1650	ara em	165		ı Gıu	166		mb LIO	Ary
Tyr Phe Asp	Asn Cvs			n Leu			Met Glv	Leu
1665	,-	1670	,		1675			1680
Ala Phe Glu	Gly Glu 168	Leu Ser	Gly As		Ile Leu	Met 1	Tyr Asn 1699	
Phe Ser Val			Ser Il			Ser I		

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_	11e 370	-	-			375	_				380	-	-		
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Ser			420					425		Asp			430		
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	Lys	515					520					525			
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	Ile			565					570					575	
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Ser	Glu	Asn	Ser 740	Ala	Thr	Glu	Ile	Gly 745	Gly	Gly	Ile	Cys	Cys 750	Lys	Glu
Ser	Leu	Glu 755	Leu	Asp	Ala	Leu	Val 760	Ser	Leu	Ser	Val	Thr 765	Glu	Asn	Leu
	Gly 770	-			-	775				-	780				
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Trp Phe Val Ser Lys Leu His Ile Thr Asp Pro Lys Glu Ala Leu Phe
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Lys Glu Lys Gly Asp Leu Ser Ile Gln Asn Phe Arg Phe Leu Ser Phe
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Thr Asp Cys Ser Ser Lys Glu Ser Ser Pro Ser Ile Ile His Gln Lys
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Asn Gly Gln Leu Ser Leu Arg Asn Asn Gly Ser Met Ser Phe Cys Arg
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Asn His Ala Glu Gly Ser Gly Gly Ala Ile Ser Ala Asp Ala Phe Ser
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Leu Gln His Asn Tyr Leu Phe Thr Ala Phe Glu Glu Asn Ser Ser Lys
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Val Ser Pro Ile Ser Phe Ala Arg Asn Arg Ala Asp Leu Asn Gly Gly
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Ala Ile Cys Cys Ser Asn Leu Ile Cys Ser Gly Asn Val Asn Pro Leu
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Phe Phe Thr Gly Asn Ser Ala Thr Asn Gly Gly Xaa Ile Cvs Cvs Ile
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Phe Ser Ser Glu Arg Leu Ser Glu Glu Glu Lys Thr Pro Asp Asn Leu
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Gln	Ala		Leu	Ile	Met	Glu		Gly	Thr	Ser	Leu		Thr	Ser	Xaa
_	_	435					440		_			445			
Asp		Lys	Leu	хаа	Thr		ser	шe	Pro	Leu		ser	Leu	Asp	Thr
a1	450		17.01	mb	T1 -	455	21.0	D		T	460	T1 -	G1	*	T1 -
465	гув	ser	Val	THE	Ile	urs	AId	PIO	ASI	475	ser	TIE	GIII	гув	480
	T-011	Car	Aen	Car	Gly	Acn	Glu	Zen	Dhe		Glu	Acn	17 a 1	Glu	
	200			485	OLI				490	-1-	014			495	200
Leu	Ser	Lvs	Glu		Asn	Asn	Ile	Pro		Leu	Thr	Leu	Pro		Glu
		•	500					505					510	•	
Gln	Ser	His	Leu	His	Leu	Pro	Asp	Gly	Asn	Leu	Ser	Ser	His	Phe	Gly
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Tyr		Gly	Asp	Trp	Thr		Ser	Trp	Lys	Asp		Asp	Glu	Gly	His
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Arg	GIn	Ser	Thr		Val	Ala	Asn	Thr		Trp	Asn	Thr	Tyr	Ser 575	Asp
Mot	CI.	77.	Ma.1	565	Ser	Mot	т1 о	n on	570	The	77.	ui a	C1		712
Mec	GIII	MIA	580	GIII	per	nec	116	585	1111	1111	мта	nro	590	GLY	міа
Tyr	Len	Phe		Thr	Trp	Glv	Ser		Val	Ser	Δen	Len		Tur	Val
-1-	200	595				,	600					605		-,-	
His	Asp		Ser	Gly	Lys	Pro		Asp	Asn	Trp	His		Arq	Ser	Leu
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Cys	Leu	Ala	Ala		Gln	Leu	Leu	Gly		Ser	Ser	Asp	Ser		Ile
	_			645		_	_		650					655	_
Thr	Ser	Thr		Thr	Thr	Ser	Tyr		Ala	Thr	Val	GIn		GIn	Leu
n1-	m)		660		*	-1-		665	a1 -	-1-	a	m	670	a1	
ALa	Inr	675	Leu	Met	Lys	TTE	680	Ala	GIN	АТА	Cys	685	Asn	GIU	ser
т1ь	Hie		T.011	Tare	Thr	Tare		Δνα	Ser	Dhe	Ser		Glu	Glv	Dha
	690	OLU	204	-,5		695		n.g	DCI	- 110	700	2,5	OLU	017	11.0
Glv		Trp	His	Ser	Val		Val	Ser	Glv	Glu		Cvs	Ala	Ser	Ile
705					710				2	715		-2-			720
Pro	Ile	Val	Ser	Asn	Gly	Ser	Gly	Leu	Phe	Ser	Ser	Phe	Ser	Ile	Phe
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Ser	Lys	Leu	Gln	Gly	Phe	Ser	Gly	Thr	Gln	Asp	Gly	Phe	Glu	Glu	Ser
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Leu		Ile	GIA	Ile	Thr		GLu	Lys	Lys	Ser		Lys	Thr	Arg	Thr
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785	Tyr	Tyr	Pne	Leu	Gly 790	ALA	Tyr	TTE	GIn	795	Leu	Lys	Arg	Asp	800
		a1	D	17.0.3	Val	т	т	T			77-3		m	3	
GIU	ser	GTA	PLO	805	Val	ьeu	ьеu	гуѕ	810	Ата	vai	ser	IIP	815	MIA
Pro	Met	Δla	Δen		Asp	Ser	Ara	Δla		Met	Dhe	Δrα	T.em		Δen
			820	Lou	р		9	825	-11		10	9	830		
Gln	Ara	Ala		His	Arg	Leu	Gln		Leu	Leu	Asn	Val		Cys	Val
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<213> Chlamydia

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Val Gln Phe Leu Arg Asn Ile Ala Asn Asp Glv Glv Ala Ile Tvr Leu 390 395 Gly Glu Ser Gly Glu Leu Ser Leu Ser Ala Asp Tyr Gly Asp Ile Ile 405 410 Phe Asp Gly Asn Leu Lys Arg Thr Ala Lys Glu Asn Ala Ala Asp Val 425 Asn Gly Val Thr Val Ser Ser Gln Ala Ile Ser Met Gly Ser Gly Gly 440 Lys Ile Thr Thr Leu Arg Ala Lys Ala Gly His Gln Ile Leu Phe Asn 455 Asp Pro Ile Glu Met Ala Asn Gly Asn Asn Gln Pro Ala Gln Ser Ser 470 475 480 Lys Leu Leu Lys Ile Asn Asp Gly Glu Gly Tyr Thr Gly Asp Ile Val 485 490 495 Phe Ala Asn Gly Ser Ser Thr Leu Tyr Gln Asn Val Thr Ile Glu Gln 500 505 510 Gly Arg Ile Val Leu Arg Glu Lys Ala Lys Leu Ser Val Asn Ser Leu 520 Ser Gln Thr Gly Gly Ser Leu Tyr Met Glu Ala Gly Ser Thr Leu Asp 535 540 Phe Val Thr Pro Gln Pro Pro Gln Gln Pro Pro Ala Ala Asn Gln Leu 550 555 Ile Thr Leu Ser Asn Leu His Leu Ser Leu Ser Ser Leu Leu Ala Asn 565 570 Asn Ala Val Thr Asn Pro Pro Thr Asn Pro Pro Ala Gln Asp Ser His 585 Pro Ala Val Ile Gly Ser Thr Thr Ala Gly Ser Val Thr Ile Ser Gly 600 605 Pro Ile Phe Phe Glu Asp Leu Asp Asp Thr Ala Tyr Asp Arg Tyr Asp 615 620 Trp Leu Gly Ser Asn Gln Lys Ile Asn Val Leu Lys Leu Gln Leu Gly 630 635 Thr Lys Pro Pro Ala Asn Ala Pro Ser Asp Leu Thr Leu Gly Asn Glu 645 650 Met Pro Lys Tyr Gly Tyr Gln Gly Ser Trp Lys Leu Ala Trp Asp Pro 665 670 Asn Thr Ala Asn Asn Gly Pro Tyr Thr Leu Lys Ala Thr Trp Thr Lys 680 Thr Gly Tyr Asn Pro Gly Pro Glu Arg Val Ala Ser Leu Val Pro Asn 695 700 Ser Leu Trp Gly Ser Ile Leu Asp Ile Arg Ser Ala His Ser Ala Ile 710 715 Gln Ala Ser Val Asp Gly Arg Ser Tyr Cys Arg Gly Leu Trp Val Ser 725 730 Gly Val Ser Asn Phe Phe Tyr His Asp Arg Asp Ala Leu Gly Gln Gly 740 745 Tyr Arg Tyr Ile Ser Gly Gly Tyr Ser Leu Gly Ala Asn Ser Tyr Phe 760 Gly Ser Ser Met Phe Gly Leu Ala Phe Thr Glu Val Phe Gly Arg Ser 775 Lys Asp Tyr Val Val Cys Arg Ser Asn His His Ala Cys Ile Gly Ser 790 795 Val Tyr Leu Ser Thr Gln Gln Ala Leu Cys Gly Ser Tyr Leu Phe Gly 805 810 815 Asp Ala Phe Ile Arg Ala Ser Tyr Gly Phe Gly Asn Gln His Met Lys 825 830 Thr Ser Tyr Thr Phe Ala Glu Glu Ser Asp Val Arg Trp Asp Asn Asn 840

Cys Leu Ala Gly Glu Ile Gly Ala Gly Leu Pro Ile Val Ile Thr Pro 855 860 Ser Lys Leu Tyr Leu Asn Glu Leu Arg Pro Phe Val Gln Ala Glu Phe 870 875 Ser Tyr Ala Asp His Glu Ser Phe Thr Glu Glu Gly Asp Gln Ala Arg 885 890 Ala Phe Lys Ser Gly His Leu Leu Asn Leu Ser Val Pro Val Gly Val 905 Lys Phe Asp Arg Cys Ser Ser Thr His Pro Asn Lys Tyr Ser Phe Met 920 925 Ala Ala Tyr Ile Cys Asp Ala Tyr Arg Thr Ile Ser Gly Thr Glu Thr 935 940 Thr Leu Leu Ser His Gln Glu Thr Trp Thr Thr Asp Ala Phe His Leu 950 955 Ala Arg His Gly Val Val Val Arg Gly Ser Met Tyr Ala Ser Leu Thr 965 970 Ser Asn Ile Glu Val Tyr Gly His Gly Arg Tyr Glu Tyr Arg Asp Ala 985 Ser Arg Gly Tyr Gly Leu Ser Ala Gly Ser Lys Val Arg Phe 1000

<210> 191

<211> 977

<212> PRT <213> Chlamydia

<400> 191

Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Ser Ser Leu 10 Val Pro Ser Ser Asp Pro His His His His His Gly Leu Ala Arg 20 25 Glu Val Pro Ser Arg Ile Phe Leu Met Pro Asn Ser Val Pro Asp Pro 40 Thr Lys Glu Ser Leu Ser Asn Lys Ile Ser Leu Thr Gly Asp Thr His Asn Leu Thr Asn Cys Tyr Leu Asp Asn Leu Arg Tyr Ile Leu Ala Ile 70 75 Leu Gln Lys Thr Pro Asn Glu Gly Ala Ala Val Thr Ile Thr Asp Tyr 90 Leu Ser Phe Phe Asp Thr Gln Lys Glu Gly Ile Tyr Phe Ala Lys Asn 105 Leu Thr Pro Glu Ser Gly Gly Ala Ile Gly Tyr Ala Ser Pro Asn Ser 115 120 125 Pro Thr Val Glu Ile Arg Asp Thr Ile Gly Pro Val Ile Phe Glu Asn 135 Asn Thr Cys Cys Arg Leu Phe Thr Trp Arg Asn Pro Tyr Ala Ala Asp 150 155 Lys Ile Arq Glu Gly Gly Ala Ile His Ala Gln Asn Leu Tyr Ile Asn 165 170 His Asn His Asp Val Val Gly Phe Met Lys Asn Phe Ser Tyr Val Gln 185 Gly Gly Ala Ile Ser Thr Ala Asn Thr Phe Val Val Ser Glu Asn Gln 195 200 205 Ser Cys Phe Leu Phe Met Asp Asn Ile Cys Ile Gln Thr Asn Thr Ala 215 220 Gly Lys Gly Gly Ala Ile Tyr Ala Gly Thr Ser Asn Ser Phe Glu Ser 230 235 Asn Asn Cys Asp Leu Phe Phe Ile Asn Asn Ala Cys Cys Ala Gly Gly

Ala Ile Phe Ser Pro Ile Cys Ser Leu Thr Gly Asn Arg Gly Asn Ile Val Phe Tyr Asn Asn Arg Cys Phe Lys Asn Val Glu Thr Ala Ser Ser Glu Ala Ser Asp Gly Gly Ala Ile Lys Val Thr Thr Arg Leu Asp Val Thr Gly Asn Arg Gly Arg Ile Phe Phe Ser Asp Asn Ile Thr Lys Asn 310 315 Tyr Gly Gly Ala Ile Tyr Ala Pro Val Val Thr Leu Val Asp Asn Gly Pro Thr Tyr Phe Ile Asn Asn Ile Ala Asn Asn Lys Gly Gly Ala Ile Tyr Ile Asp Gly Thr Ser Asn Ser Lys Ile Ser Ala Asp Arg His Ala Ile Ile Phe Asn Glu Asn Ile Val Thr Asn Val Thr Asn Ala Asn Gly Thr Ser Thr Ser Ala Asn Pro Pro Arg Arg Asn Ala Ile Thr Val Ala Ser Ser Ser Gly Glu Ile Leu Leu Gly Ala Gly Ser Ser Gln Asn Leu Ile Phe Tyr Asp Pro Ile Glu Val Ser Asn Ala Gly Val Ser Val Ser Phe Asn Lys Glu Ala Asp Gln Thr Gly Ser Val Val Phe Ser Gly Ala Thr Val Asn Ser Ala Asp Phe His Gln Arg Asn Leu Gln Thr Lys Thr Pro Ala Pro Leu Thr Leu Ser Asn Gly Phe Leu Cys Ile Glu Asp His Ala Gln Leu Thr Val Asn Arg Phe Thr Gln Thr Gly Gly Val Val Ser Leu Gly Asn Gly Ala Val Leu Ser Cys Tyr Lys Asn Gly Thr Gly Asp Ser Ala Ser Asn Ala Ser Ile Thr Leu Lys His Ile Gly Leu Asn Leu Ser Ser Ile Leu Lys Ser Gly Ala Glu Ile Pro Leu Leu Trp Val Glu Pro Thr Asn Asn Ser Asn Asn Tyr Thr Ala Asp Thr Ala Ala Thr Phe Ser Leu Ser Asp Val Lys Leu Ser Leu Ile Asp Asp Tyr Gly Asn Ser Pro Tyr Glu Ser Thr Asp Leu Thr His Ala Leu Ser Ser Gln Pro Met Leu Ser Ile Ser Glu Ala Ser Asp Asn Gln Leu Gln Ser Glu Asn Ile Asp Phe Ser Gly Leu Asn Val Pro His Tyr Gly Trp Gln Gly Leu Trp Thr Trp Gly Trp Ala Lys Thr Gln Asp Pro Glu Pro Ala Ser Ser Ala Thr Ile Thr Asp Pro Gln Lys Ala Asn Arg Phe His Arg Thr Leu Leu Leu Thr Trp Leu Pro Ala Gly Tyr Val Pro Ser Pro Lys His Arg Ser Pro Leu Ile Ala Asn Thr Leu Trp Gly Asn Met Leu Leu Ala Thr Glu Ser Leu Lys Asn Ser Ala Glu Leu Thr Pro Ser Gly His Pro Phe Trp Gly Ile Thr Gly Gly Gly Leu Gly Met Met Val Tyr Gln Asp Pro Arg

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710
                                  715
Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr Ser Ala Gly
             725
                     730 735
Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe Ser Gln Thr
                   745
Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val Ser Ser Lys
                         760
                              765
Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln Glu Gly Phe
                    775
                           780
Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp His Asn Cys
                790
                                   795
His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln Gly Thr Phe
             805
                               810
Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu Pro Met Lys
                            825
Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu Gly Ala Leu
                        840
Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly Ala Tyr Pro
                    855
Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu Val Pro Ile
                                   875
               870
Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro Gln Ala Trp
              885
                                890
Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln Glu Pro Gly
                            905
Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe Gly Ser Gly
                        920
Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser Gln Gln Thr
                     935
Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His Gly Phe Tyr
                 950
                                   955
Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile Ala Leu Arg
              965
                                970
Phe
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<210> 192 <211> 848

<212> PRT

<213> Chlamydia

<400> 192

Met Ala Ser His His His His His Gly Ala Ile Ser Cys Leu Arg 10 Gly Asp Val Val Ile Ser Gly Asn Lys Gly Arg Val Glu Phe Lys Asp 25 Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu Thr Val Glu Lys Val Glu 40 Glu Val Glu Pro Ala Pro Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe 55 Leu Gly Ser Val Glu Gln Ser Phe Ile Thr Ala Ala Asn Gln Ala Leu 75 70 Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser 90 Glu Glu Leu Ala Lys Arg Arg Glu Cys Ala Gly Gly Ala Ile Phe Ala 105 Lys Arg Val Arg Ile Val Asp Asn Gln Glu Ala Val Val Phe Ser Asn 115 120

Asn Phe Ser Asp Ile Tyr Gly Gly Ala Ile Phe Thr Gly Ser Leu Arg 135 Glu Glu Asp Lys Leu Asp Gly Gln Ile Pro Glu Val Leu Ile Ser Gly 155 Asn Ala Gly Asp Val Val Phe Ser Gly Asn Ser Ser Lys Arg Asp Glu 165 170 His Leu Pro His Thr Gly Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr 185 Ile Ser Gln Asn Thr Gly Asn Val Leu Phe Tyr Asn Asn Val Ala Cys 200 Ser Gly Gly Ala Val Arg Ile Glu Asp His Gly Asn Val Leu Leu Glu 215 220 Ala Phe Gly Gly Asp Ile Val Phe Lys Gly Asn Ser Ser Phe Arg Ala 230 235 Gln Gly Ser Asp Ala Ile Tyr Phe Ala Gly Lys Glu Ser His Ile Thr 245 250 Ala Leu Asn Ala Thr Glu Gly His Ala Ile Val Phe His Asp Ala Leu 265 Val Phe Glu Asn Leu Lys Glu Arg Lys Ser Ala Glu Val Leu Leu Ile 280 Asn Ser Arg Glu Asn Pro Gly Tyr Thr Gly Ser Ile Arg Phe Leu Glu 295 300 Ala Glu Ser Lys Val Pro Gln Cys Ile His Val Gln Gln Gly Ser Leu 310 315 Glu Leu Leu Asn Gly Ala Thr Leu Cys Ser Tyr Gly Phe Lys Gln Asp 325 330 Ala Gly Ala Lys Leu Val Leu Ala Ala Gly Ser Lys Leu Lys Ile Leu 340 345 Asp Ser Gly Thr Pro Val Gln Gly His Ala Ile Ser Lys Pro Glu Ala 360 365 Glu Ile Glu Ser Ser Ser Glu Pro Glu Gly Ala His Ser Leu Trp Ile 375 380 Ala Lys Asn Ala Gln Thr Thr Val Pro Met Val Asp Ile His Thr Ile 390 3 9 5 Ser Val Asp Leu Ala Ser Phe Ser Ser Ser Gln Glu Gly Thr Val 405 410 Glu Ala Pro Gln Val Ile Val Pro Gly Gly Ser Tyr Val Arg Ser Gly 425 430 Glu Leu Asn Leu Glu Leu Val Asn Thr Thr Gly Thr Gly Tyr Glu Asn 440 His Ala Leu Leu Lys Asn Glu Ala Lys Val Pro Leu Met Ser Phe Val 455 Ala Ser Ser Asp Glu Ala Ser Ala Glu Ile Ser Asn Leu Ser Val Ser 470 475 Asp Leu Gln Ile His Val Ala Thr Pro Glu Ile Glu Glu Asp Thr Tyr 490 Gly His Met Gly Asp Trp Ser Glu Ala Lys Ile Gln Asp Gly Thr Leu 505 Val Ile Asn Trp Asn Pro Thr Gly Tyr Arg Leu Asp Pro Gln Lys Ala 520 Gly Ala Leu Val Phe Asn Ala Leu Trp Glu Glu Gly Ala Val Leu Ser 535 540 Ala Leu Lys Asn Ala Arg Phe Ala His Asn Leu Thr Ala Gln Arg Met 550 555 Glu Phe Asp Tyr Ser Thr Asn Val Trp Gly Phe Ala Phe Gly Gly Phe 565 570 Arg Thr Leu Ser Ala Glu Asn Leu Val Ala Ile Asp Gly Tyr Lys Gly 580 585

Ala Tyr Gly Gly Ala Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp 600 Phe Val Leu Gly Val Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser 615 620 Gln Lys Phe Asp Ala Glu Val Ser Arg Lys Glý Val Val Gly Ser Val 630 635 Tyr Thr Gly Phe Leu Ala Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser 645 650 Leu Gly Glu Thr Gln Asn Asp Met Lys Thr Arg Tyr Gly Val Leu Gly 665 Glu Ser Ser Ala Ser Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu 680 Val Glu Tyr Arg Ser Leu Val Gly Pro Val Arg Pro Thr Phe Tyr Ala 695 700 Leu His Phe Asn Pro Tyr Val Glu Val Ser Tyr Ala Ser Met Lys Phe 710 715 Pro Gly Phe Thr Glu Gln Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala 725 730 Ser Leu Thr Asn Ile Thr Ile Pro Leu Gly Met Lys Phe Glu Leu Ala 745 Phe Ile Lys Gly Gln Phe Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr 755 760 765 Ala Trp Glu Ala Tyr Arg Lys Val Glu Gly Gly Ala Val Gln Leu Leu 775 780 Glu Ala Gly Phe Asp Trp Glu Gly Ala Pro Met Asp Leu Pro Arg Gln 790 795 Glu Leu Arg Val Ala Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe 810 Ser Thr Val Leu Gly Leu Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr 825 Asp Ser Lys Leu Gly Tyr Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 835 840

<400> 193

Met His His His His His Gly Leu Ala Ser Cys Val Asp Leu His 10 Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val Gly Pro Gln Ala Val Leu Leu Leu Asp Gln Ile Arg Asp Leu Phe Val Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly Asp Pro Ser Ser 55 Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys Val Glu Gln Ser 70 75 Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln Gly Val Asp Gln 85 90 Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser Phe Thr Ser Ser 105 Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu Gly Ile Ala Phe 120 Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr Asp Val Lys Ala 135 140 Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp Leu Ile Phe Glu

<sup>&</sup>lt;210> 193

<sup>&</sup>lt;211> 778 <212> PRT

<sup>&</sup>lt;213> Chlamydia

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150
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Lys Ile Lys Gly Gly Leu Glu Phe Ala Ser Cys Ser Ser Leu Glu Gln
             165
                     170
Gly Gly Ala Cys Ala Ala Gln Ser Ile Leu Ile His Asp Cys Gln Gly
                   185
Leu Gln Val Lys His Cys Thr Thr Ala Val Asn Ala Glu Gly Ser Ser
                       200
Ala Asn Asp His Leu Gly Phe Gly Gly Gly Ala Phe Phe Val Thr Gly
                    215
                                       220
Ser Leu Ser Gly Glu Lys Ser Leu Tyr Met Pro Ala Gly Asp Met Val
                 230 235
Val Ala Asn Cys Asp Gly Ala Ile Ser Phe Glu Gly Asn Ser Ala Asn
              245
                                250
Phe Ala Asn Gly Gly Ala Ile Ala Ala Ser Gly Lys Val Leu Phe Val
           260
                            265
Ala Asn Asp Lys Lys Thr Ser Phe Ile Glu Asn Arg Ala Leu Ser Gly
       275
                         280
                                           285
Gly Ala Ile Ala Ala Ser Ser Asp Ile Ala Phe Gln Asn Cys Ala Glu
                     295
Leu Val Phe Lys Gly Asn Cys Ala Ile Gly Thr Glu Asp Lys Gly Ser
                  310
                                    315
Leu Gly Gly Gly Ala Ile Ser Ser Leu Gly Thr Val Leu Leu Gln Gly
              325
                                330
Asn His Gly Ile Thr Cys Asp Lys Asn Glu Ser Ala Ser Gln Gly Gly
           340
                            345
Ala Ile Phe Gly Lys Asn Cys Gln Ile Ser Asp Asn Glu Gly Pro Val
                         360
                                           365
Val Phe Arg Asp Ser Thr Ala Cys Leu Gly Gly Gly Ala Ile Ala Ala
                     375
                                        380
Gln Glu Ile Val Ser Ile Gln Asn Asn Gln Ala Gly Ile Ser Phe Glu
                  390
                                    395
Gly Gly Lys Ala Ser Phe Gly Gly Gly Ile Ala Cys Gly Ser Phe Ser
              405
                                410
Ser Ala Gly Gly Ala Ser Val Leu Gly Thr Ile Asp Ile Ser Lys Asn
           420
                            425
Leu Gly Ala Ile Ser Phe Ser Arg Thr Leu Cys Thr Thr Ser Asp Leu
                       440
Gly Gln Met Glu Tyr Gln Gly Gly Gly Ala Leu Phe Gly Glu Asn Ile
                     455
Ser Leu Ser Glu Asn Ala Gly Val Leu Thr Phe Lys Asp Asn Ile Val
                 470
                                    475
Lys Thr Phe Ala Ser Asn Gly Lys Ile Leu Gly Gly Gly Ala Ile Leu
                                490
Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly Gly Ile Ser Phe
           500
                             505
Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr Gln Glu Glu Phe
                         520
Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser Ser Gly Tyr Ser
Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile Leu His Asn Ala
                                    555
Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser Glu Glu Glu Ala
                                570
              565
Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His Gly Met Asp Ser
                            585
Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly Asn Asn Tyr Ala
                         600
Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser Lys Thr Val Gln
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615
                                      620
Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn Ile Ala Ser Leu
                630
                         635
Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys Glu Leu Val Asp
                   650
              645
Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly
                           665
Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser Gly Asn Lys Gly
                       680
Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu
                  695
Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro Glu Gln Lys Asp
              710 715
Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln Ser Phe Ile Thr
             725
                             730 735
Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro
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Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg Arg Glu Cys Ala
                       760
Gly Gly Ala Asp Ser Ser Arg Ser Gly Cys
                    775
<210> 194
<211> 948
<212> PRT
<213> Chlamydia
<400> 194
Met Ala Ser Met His His His His His His Val Lys Ile Glu Asn Phe
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Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala Ile Asp Asn Thr Thr
                           25
Glu Gly Ser Ser Lys Ser Asn Val Leu Gly Gly Ala Val Tyr Ala
                       40
Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser Arg Arg Thr Val Thr
                   55
Phe Ser Gly Asn Thr Val Ser Ser Gln Ser Thr Thr Gly Gln Val Ala
               70
Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile Ala Thr Pro Val Val
             85
                              90
Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr
                           105
Gln Arg Lys Asp Thr Phe Gly Gly Ala Ile Gly Ala Thr Ser Ala Val
                        120
Ser Leu Ser Gly Gly Ala His Phe Leu Glu Asn Val Ala Asp Leu Gly
                    135
                                     140
Ser Ala Ile Gly Leu Val Pro Asp Thr Gln Asn Thr Glu Thr Val Lys
                 150
                                  155
Leu Glu Ser Gly Ser Tyr Tyr Phe Glu Lys Asn Lys Ala Leu Lys Arg
                              170
             165
Ala Thr Ile Tyr Ala Pro Val Val Ser Ile Lys Ala Tyr Thr Ala Thr
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185 Phe Asn Gln Asn Arg Ser Leu Glu Glu Gly Ser Ala Ile Tyr Phe Thr 200

235

Lys Glu Ala Ser Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn

Leu Val Thr Pro Thr Leu Ser Thr Thr Thr Glu Gly Thr Pro Ala Thr

205

180

210 215 220

Thr	Ser	Gly	Asp	Val 245	Thr	Lys	Tyr	Gly	Ala 250	Ala	Ile	Phe	Gly	Gln 255	Ile
Ala	ser	Ser	Asn 260		Ser	Gln	Thr	Asp 265		Leu	Pro	Leu	Lys 270		Ile
Ala	ser	Gly 275	Gly	Asn	Ile	Cys	Phe 280	Arg	Asn	Asn	Glu	Tyr 285	Arg	Pro	Thr
Ser	Ser 290	Asp	Thr	Gly	Thr	Ser 295		Phe	Cys	Ser	Ile 300	Ala	Gly	Asp	Val
305					310				-	315		Ser			320
Ala	Ile	Arg	Thr	Ser 325	Thr	Lys	Lys	Thr	Gly 330	Thr	Gln	Ala	Thr	Ala 335	Tyr
Asp	Thr	Leu	Asp 340	Ile	Asn	Lys	Ser	Glu 345	Asp	Ser	Glu	Thr	Val 350	Asn	Ser
Ala	Phe	Thr 355	Gly	Thr	Ile	Leu	Phe 360	Ser	Ser	Glu	Leu	His 365	Glu	Asn	Lys
Ser	370					375					380	Ser			
385					390					395		Gln			400
				405					410			Asn		415	
	-		420					425				Asp	430		
		435			Ile		440					445	Pro		
	450					455					460	Gly			
465					470				_	475		Lys			480
				485					490			Gly		495	
			500					505				Asn Thr	510		
	Asn	515					520					525 Ile			
	530					535					540	Gln			
545					550					555		Ala			560
				565		_			570			Thr		575	
			580					585				Ser	590		
		595					600					605 Arg			
	610					615					620	Met			
625					630					635		Arg			640
Val.				645		_			650			Thr		655	
Glr.			660					665					670		
		675					680					Tyr 685			
wra	690	val	мта	Leu	мыр	695	гÀR	PLO	мта	птв	700	Val	тте	vall	GΙΫ

Ala Ala Phe Ser Lys Met Ile Gly Lys Thr Lys Ser Leu Lys Arg Glu 710 715 Asn Asn Tyr Thr His Lys Gly Ser Glu Tyr Ser Tyr Gln Ala Ser Val 725 730 Tyr Gly Gly Lys Pro Phe His Phe Val Ile Asn Lys Lys Thr Glu Lys 745 Ser Leu Pro Leu Leu Gln Gly Val Ile Ser Tyr Gly Tyr Ile Lys 760 765 His Asp Thr Val Thr His Tyr Pro Thr Ile Arg Glu Arg Asn Gln Gly 775 780 Glu Trp Glu Asp Leu Gly Trp Leu Thr Ala Leu Arg Val Ser Ser Val 790 795 Leu Arg Thr Pro Ala Gln Gly Asp Thr Lys Arg Ile Thr Val Tyr Gly 805 810 Glu Leu Glu Tyr Ser Ser Ile Arg Gln Lys Gln Phe Thr Glu Thr Glu 820 825 Tyr Asp Pro Arg Tyr Phe Asp Asn Cys Thr Tyr Arg Asn Leu Ala Ile 840 Pro Met Gly Leu Ala Phe Glu Gly Glu Leu Ser Gly Asn Asp Ile Leu 855 860 Met Tyr Asn Arg Phe Ser Val Ala Tyr Met Pro Ser Ile Tyr Arg Asn 870 875 Ser Pro Thr Cys Lys Tyr Gln Val Leu Ser Ser Gly Glu Gly Glu 885 890 Ile Ile Cys Gly Val Pro Thr Arg Asn Ser Ala Arg Gly Glu Tyr Ser 900 905 Thr Gln Leu Tyr Pro Gly Pro Leu Trp Thr Leu Tyr Gly Ser Tyr Thr 920 925 Ile Glu Ala Asp Ala His Thr Leu Ala His Met Met Asn Cys Gly Ala 930 935 940 Arg Met Thr Phe 945

<210> 195

<211> 821

<212> PRT

<213> Chlamydia

<400> 195

Met His His His His His Glu Ala Ser Ser Ile Gln Asp Gln Ile 10 Lys Asn Thr Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln 20 25 Ala Phe Thr Asp Met Met Leu Ala Asp Asn Thr Glu Tyr Arg Ala Ala Asp Ser Val Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arq 55 Lys His Leu Ser Ser Ser Glu Ala Ser Pro Thr Thr Glu Gly Val 70 75 Ser Ser Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala 90 Pro Ser Ser Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn 100 105 Gly Gly Ile Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln 120 125 Asp Ser Leu Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe 135 140 Phe Gly Glu Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn

145					150					155					160
Gly	Gly	Ala	Ile	Tyr 165	Gly	Glu	Lys	Glu	Val 170		Phe	Glu	Asn	Ile 175	Lys
Ser	Leu	Leu	Val 180	Glu	Val	Asn	Ile	Ser 185		Glu	Lys	Gly	Gly 190	Ser	Val
Tyr	Ala	Lys 195	Glu	Arg	Va1	ser	Leu 200	Glu	Asn	Val	Thr	Glu 205	Ala	Thr	Phe
Ser	Ser 210	Asn	Gly	Gly	Glu	Gln 215	Gly	Gly	Gly	Gly	11e 220	Tyr	Ser	Glu	Gln
Asp 225	Met	Leu	Ile	ser	Asp 230	Cys	Asn	Asn	Val	His 235	Phe	Gln	Gly	Asn	Ala 240
Ala	Gly	Ala	Thr	Ala 245	Val	Lys	Gln	Cys	Leu 250		Glu	Glu	Met	11e 255	Val
Leu	Leu	Thr	Glu 260	Cys	Val	Asp	Ser	Leu 265	Ser	Glu	Asp	Thr	Leu 270	Asp	Ser
Thr	Pro	Glu 275	Thr	Glu	Gln	Thr	Lys 280	Ser	Asn	Gly	Asn	Gln 285	Asp	Gly	Ser
	290		_	_	Thr	295					300				
3 0 5					Leu 310					315					320
				325	Gly				330					335	
			340		Gly		Ī	345			_		350		
		355			Ser		360			-		365		_	
	370	-	-		Tyr	375					380				
385					Thr 390					395	-				400
				405	Lys	-			410			-		415	
			420		Leu	-		425				-	430		
		435			Ala		440					445			
	450				Ser Ala	455					460				
465	FIO	GIU	Val	val	470	361	MIG	Буь	116	475	Arg	FILE	FIIC	MIG	480
Thr	Ala	Glu	Pro	Ala 485	Ala	Pro	Ser	Leu	Thr 490	Glu	Ala	Glu	Ser	Asp 495	Gln
Thr	Asp	Gln	Thr 500	Glu	Thr	Ser	Asp	Thr 505	Asn	Ser	Asp	Ile	Asp 510	Val	Ser
Ile	Glu	Asn 515	Ile	Leu	Asn	Val	Ala 520	Ile	Asn	Gln	Asn	Thr 525	Ser	Ala	Lys
	530				Tyr	535					540				
Asn 545	Leu	Glu	Leu	Ser	Gly 550	Asn	Ser	Ser	Gln	Asp 555	Val	Gly	Gly	Gly	Leu 560
				565	Val				570					575	
			580		Ala			585					590		
		595			Leu	-	600					605	-		
Val	Lys	Ala	Ile	Val	Glu	ser	Thr	Pro	Glu	Ala	Pro	Glu	Glu	Ile	Pro

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615
Pro Val Glu Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn
       630 635 640
Thr Glu Gly Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp
            645
                   650
Thr Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr
                665 670
Ser Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr
             680 685
Gln Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser
         695 700
Asn Glu Asn Thr Asp Glu Ser Ser Asp Ser His Thr Glu Glu Ile Thr
                710
                                 715
Asp Glu Ser Val Ser Ser Ser Ser Lys Ser Gly Ser Ser Thr Pro Gln
            725
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Asp Gly Gly Ala Ala Ser Ser Gly Ala Pro Ser Gly Asp Gln Ser Ile
         740
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Ser Ala Asn Ala Cys Leu Ala Lys Ser Tyr Ala Ala Ser Thr Asp Ser
                      760
Ser Pro Val Ser Asn Ser Ser Gly Ser Asp Val Thr Ala Ser Ser Asp
       775
Asn Pro Asp Ser Ser Ser Ser Gly Asp Ser Ala Gly Asp Ser Glu Gly
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Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
               70
                                75
Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
                             90
Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
                         105
         100
Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
                     120 125
     115
Leu Ala Glu Gly Pro Pro Ala Glu Phe Pro Leu Val Pro Arg Gly Ser
                  135 140
Pro Leu Pro Val Gly Asn Pro Ala Glu Pro Ser Leu Leu Ile Asp Gly
               150 155
Thr Met Trp Glu Gly Ala Ser Gly Asp Pro Cys Asp Pro Cys Ala Thr
          165 170
Trp Cys Asp Ala Ile Ser Ile Arg Ala Gly Tyr Tyr Gly Asp Tyr Val
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105

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Phe Asp Arg Val Leu Lys Val Asp Val Asn Lys Thr Phe Ser Gly Met
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Ala Ala Thr Pro Thr Gln Ala Ile Gly Asn Ala Ser Asn Thr Asn Gln
                      215
                                          220
Pro Glu Ala Asn Gly Arg Pro Asn Ile Ala Tyr Gly Arg His Met Gln
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                   230
Asp Ala Glu Trp Phe Ser Asn Ala Ala Phe Leu Ala Leu Asn Ile Trp
               245
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Asp Arg Phe Asp Ile Phe Cys Thr Leu Gly Ala Ser Asn Gly Tyr Phe
                                                 270
           260
                              265
Lys Ala Ser Ser Ala Ala Phe Asn Leu Val Gly Leu Ile Gly Phe Ser
       275
                           280
                                              285
Ala Ala Ser Ser Ile Ser Thr Asp Leu Pro Met Gln Leu Pro Asn Val
                       295
                                          300
Gly Ile Thr Gln Gly Val Val Glu Phe Tyr Thr Asp Thr Ser Phe Ser
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                                      315
Trp Ser Val Gly Ala Arg Gly Ala Leu Trp Glu Cys Gly Cys Ala Thr
               325
                                  330
Leu Gly Ala Glu Phe Gln Tyr Ala Gln Ser Asn Pro Lys Ile Glu Met
           340
                              345
                                                  350
Leu Asn Val Thr Ser Ser Pro Ala Gln Phe Val Ile His Lys Pro Arg
       355
                           360
                                              365
Gly Tyr Lys Gly Ala Ser Ser Asn Phe Pro Leu Pro Ile Thr Ala Gly
   370
                       375
                                           380
Thr Thr Glu Ala Thr Asp Thr Lys Ser Ala Thr Ile Lys Tyr His Glu
                   390
                                       395
Trp Gln Val Gly Leu Ala Leu Ser Tyr Arg Leu Asn Met Leu Val Pro
                                  410
Tyr Ile Gly Val Asn Trp Ser Arg Ala Thr Phe Asp Ala Asp Thr Ile
           420
                              425
                                                  430
Arg Ile Ala Gln Pro Lys Leu Lys Ser Glu Ile Leu Asn Ile Thr Thr
                          440
Trp Asn Pro Ser Leu Ile Gly Ser Thr Thr Ala Leu Pro Asn Asn Ser
                       455
Gly Lys Asp Val Leu Ser Asp Val Leu Gln Ile Ala Ser Ile Gln Ile
                  470
                                      475
Asn Lys Met Lys Ser Arg Lys Ala Cys Gly Val Ala Val Gly Ala Thr
               485
                                  490
Leu Ile Asp Ala Asp Lys Trp Ser Ile Thr Gly Glu Ala Arg Leu Ile
           500
                              505
Asn Glu Arg Ala Ala His Met Asn Ala Gln Phe Arg Phe
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                           520
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cagaacgcgt ttagaatgtc atacgagcac cgca

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<sup>&</sup>lt;210> 198

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Val Pro His His His His His
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Pro Met Pro Arg
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Glu Ile Val Lys
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Val Trp Glu Tyr
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Lys Lys His Asn
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Pro Asp Ala Asn
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Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn
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Leu Ala Lys Val
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<210> 232
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Ile Asp Met Phe
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<212> PRT

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Ser Lys His Ile Val Lys
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Tyr Pro Val Glu
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  Val Ile Ile Thr
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  Ala Glu Phe Val
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 Ser Asp Pro Ala
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Thr Thr Pro Thr
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Asp Gly Lys Leu
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Val Arg Ser Asp Pro Ala Thr Thr Pro Thr Ala Asp Gly Lys Leu Val
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Trp Lys Ile Asp
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<220>
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                                   10
 Leu Gly Gln Gly
            20
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Ala Asp Gly Lys Leu Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu
 1
Lys Ser Lys Ile
            20
<210> 248
<211> 20
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<223> Made in a lab
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Val Trp Lys Ile Asp Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr
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                                    10
Val Trp Val Lys
            20
<210> 249
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Arg Leu Gly Gln Gly Glu Lys Ser Lys Ile Thr Val Trp Val Lys Pro
                                    10
 1
Leu Lys Glu Gly
            20
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                                  10
Cys Cys Phe Thr
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Lys Ile Thr Val Trp Val Lys Pro Leu Lys Glu Gly
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Phe Gly Val Leu
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Pro Glu Gly Ser
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                                    10
                                                        15
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Phe Leu Ile Asp
            20
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His Gly Val Ile
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<212> PRT
<213> Artificial Sequence
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<220>
 <223> Made in a lab
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 1
 His Ala Val Ile
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                                    10
 Asp Leu Pro Leu
            20
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<400> 261
Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu Pro Leu Gly
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                                   10
Arg Ser Ile Asp
            20
<210> 262
<211> 20
<212> PRT
<213> Artificial Sequence
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Glu Leu Arg Ile
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<211> 897
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120

180

240

300

360

420

480

540

600

660

720

780

840

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attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
gegggetett eegeacacat tacagettee caagtgteea aaggattagg ggatgegaga
actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
caaagettet teteteacat gaaagetget agteagaaaa egcaagaagg ggatgagggg
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
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agetatatta tggcqgctaa ccatqcaqcq tetgtggtqq qtqctqqact cqctatcaqt
gognaaagag cagattgoga agooogotgo gotogtattg ogagagaaga gtogttacto
gaagtgccgg gagaggaaaa tgcttgcgag aagaaagtcg ctggagagaa agccaagacg
ttcacgcgca tcaagtatgc actcctcact atgctcgaga agtttttgga atgcgttgcc
gacgttttca aattggtgcc gctgcctatt acaatgggta ttcgtgcgat tgtggctgct
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<221> VARIANT
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Lys Thr Lys Gly Val Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
Glu Leu Thr Ala Asn Ile Leu Glu Gln Ala Gly Gly Ala Gly Ser Ser
Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Ala Arg
                    70
                                        75
Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr
Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln
                                                    110
Lys Thr Gln Glu Gly Asp Glu Gly Leu Thr Ala Asp Leu Cys Val Ser
                            120
                                                125
His Lys Arq Arq Ala Ala Ala Val Cys Ser Ile Ile Gly Gly Ile
    130
                        135
                                            140
Thr Tyr Leu Ala Thr Phe Gly Ala Ile Arg Pro Ile Leu Phe Val Asn
                    150
                                        155
Lys Met Leu Ala Lys Pro Phe Leu Ser Ser Gln Thr Lys Ala Asn Met
                165
                                    170
                                                        175
Gly Ser Ser Val Ser Tyr Ile Met Ala Ala Asn His Ala Ala Ser Val
                                185
                                                    190
Val Gly Ala Gly Leu Ala Ile Ser Ala Xaa Arg Ala Asp Cys Glu Ala
                            200
                                                205
Arg Cys Ala Arg Ile Ala Arg Glu Glu Ser Leu Leu Glu Val Pro Gly
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55

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215
Glu Glu Asn Ala Cys Glu Lys Lys Val Ala Gly Glu Lys Ala Lys Thr
                    230
                                        235
Phe Thr Arg Ile Lys Tyr Ala Leu Leu Thr Met Leu Glu Lys Phe Leu
                245
                                    250
Glu Cys Val Ala Asp Val Phe Lys Leu Val Pro Leu Pro Ile Thr Met
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Gly Ile Arg Ala Ile Val Ala Ala Gly Cys Thr Phe Thr Ser Ala Ile
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Ile Gly Leu Cys Thr Phe Cys Ala Arg Ala
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                                                                      120
attaaggttg ccaagtctgc tgccgaattg accgcaaata ttttggaaca agctggaggc
                                                                      180
gegggetett eegeacacat tacagettee caagtgteea aaggattagg ggatgegaga
                                                                       240
actgttgtcg ctttagggaa tgcctttaac ggagcgttgc caggaacagt tcaaagtgcg
                                                                       300 -
caaagettet teteteacat gaaagetget agteagaaaa egcaagaagg ggatgagggg
                                                                      360
ctcacagcag atctttgtgt gtctcataag cgcagagcgg ctgcggctgt ctgtagcatc
                                                                      420
ateggaggaa ttacctacct egegacatte ggagetatee gteegattet gtttgtcaae
                                                                      480
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Lys Thr Lys Gly Met Asp Lys Thr Ile Lys Val Ala Lys Ser Ala Ala
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PCT/US00/32919

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		ttcttgaata gtaggcgcac				420
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Ile Phe Phe Glu Asn His Gly Met Val Cys Pro Ala Asn Trp Arg Ser \$165\$ \$170\$ \$175\$

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Pro Leu Pro Thr Ala Ser Cys Val Glu Thr Lys Ser Thr Ser Ser Ser 20 25 30

Thr Lys Gly Asn Thr Cys Ser Lys Ile Leu Asp Ile Ala Leu Ala Ile 35 40 45

Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys 50 60

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile 65 70 75 80

Gly Ser Ala Cys Val Gly Ala Gly Ile Ser Arg Leu Met Tyr Arg Ser 85 90 95

Ser Tyr Ala Ser Leu Glu Ala Lys Asn Val Leu Ala Glu Gln Arg Leu 100 105 110

Arg Asn Leu Ser Glu Glu Lys Asp Ala Leu Ala Ser Val Ser Phe Ile 115 \$120\$

Asn Lys Met Phe Leu Arg Gly Leu Thr Asp Asp Leu Gln Ala Leu Glu 130 135 140

Ala Lys Val Met Glu Phe Glu Ile Asp Cys Leu Asp Arg Leu Glu Lys 145 \$150\$

Asn Glu Gln Ala Leu Leu Ser Asp Val Arg Leu Val Leu Ser Ser Tyr 165 170 175

Thr Arg Trp Leu Asp 180

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Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu Ser Ala Tyr Leu Pro Tyr Thr Val Gln Lys Ser Ser Asp Gly Arg Phe

135

Tyr 145	Phe	Val	Asp	Ile	Met 150	Thr	Phe	Ser	Ser	Glu 155	Ile	Arg	Val	Gly	Asp 160
Glu	Leu	Leu	Glu	Val 165	Asp	Gly	Ala	Pro	Val 170	Gln	Asp	Val	Leu	Ala 175	Thr
Leu	Tyr	Gly	Ser 180	Asn	His	Lys	Gly	Thr 185	Ala	Ala	Glu	Glu	Ser 190	Ala	Ala
Leu	Arg	Thr 195	Leu	Phe	Ser	Arg	Met 200	Ala	Ser	Leu	Gly	His 205	Lys	Val	Pro
Ser	Gly 210	Arg	Thr	Thr	Leu	Lys 215	Ile	Arg	Arg	Pro	Phe 220	Gly	Thr	Thr	Arg
Glu 225	Val	Arg	Val	Lys	Trp 230	Arg	Tyr	Val	Pro	Glu 235	Gly	Val	Gly	Asp	Leu 240
Ala	Thr	Ile	Ala	Pro 245	Ser	Ile	Arg	Ala	Pro 250	Gln	Leu	Gln	Lys	Ser 255	Met
Arg	Ser	Phe	Phe 260	Pro	Lys	Lys	Asp	Asp 265	Ala	Phe	His	Arg	Ser 270	Ser	Ser
Leu	Phe	Tyr 275	Ser	Pro	Met	Val	Pro 280	His	Phe	Trp	Ala	Glu 285	Leu	Arg	Asn
His	Tyr 290	Ala	Thr	Ser	Gly	Leu 295	Lys	Ser	Gly	Tyr	Asn 300	Ile	Gly	Ser	Thr
Asp 305	Gly	Phe	Leu	Pro	Val 310	Ile	Gly	Pro	Val	Ile 315	Trp	Glu	Ser	Glu	Gly 320
Leu	Phe	Arg	Ala	Tyr 325	Ile	Ser	Ser	Val	Thr 330	Asp	Gly	Asp	Gly	Lys 335	Ser
His	Lys	Val	Gly 340	Phe	Leu	Arg	Ile	Pro 345	Thr	Tyr	Ser	Trp	Gln 350	Asp	Met
Glu	Asp	Phe 355	Asp	Pro	Ser	Gly	Pro 360	Pro	Pro	Trp	Glu	Glu 365	Phe	Ala	Lys
Ile	Ile 370	Gln	Val	Phe	Ser	Ser 375	Asn	Thr	Glu	Ala	Leu 380	Ile	Ile	Asp	Gln
Thr 385	Asn	Asn	Pro	Gly	Gly 390	Ser	Val	Leu	Tyr	Leu 395	Tyr	Ala	Leu	Leu	Ser 400
Met	Leu	Thr	Asp	Arg 405	Pro	Leu	Glu	Leu	Pro 410	Lys	His	Arg	Met	Ile 415	Leu
Thr	Gln	Asp	Glu 420	Val	Val	Asp	Ala	Leu 425	Asp	Trp	Leu	Thr	Leu 430	Leu	Glu
Asn	Val	Asp 435	Thr	Asn	Val	Glu	Ser 440	Arg	Leu	Ala		Gly 445	Asp	Asn	Met

Glu Gly Tyr Thr Val Asp Leu Gln Val Ala Glu Tyr Leu Lys Ser Phe 450 455 460

Gly Arg Gln Val Leu Asn Cys Trp Ser Lys Gly Asp Ile Glu Leu Ser 465 470 475 480

Thr Pro Ile Pro Leu Phe Gly Phe 485

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<211> 140 <212> PRT

<213> Chlamydia

<213> Chiamydia

<400> 298

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Val Asn Ala Leu Thr Tyr Ser His Val Leu Arg Asp Leu Ser Val Ser 20 25 30

Met Asp Ala Leu Phe Ser Arg Asn Thr Leu Ala Val Leu Leu Gly Leu 35 40 45

Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly 50 60

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr 65 70 75 80

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly 85 90 95

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys 100 105 110

His Ala Ser Trp Ile Ala Leu Ala Ser Tyr Phe Gly Gly Leu Ala Val 115 120 125

Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val 130 135 140

<210> 299

<211> 361

<212> PRT

<213> Chlamydia

<400> 299

His Gln Glu Ile Ala Asp Ser Pro Leu Val Lys Lys Ala Glu Gln Gln 5 10 15

Ile Asn Gln Ala Gln Gln Asp Ile Gln Thr Ile Thr Pro Ser Gly Leu 20 25 30

Asp Ile Pro Ile Val Gly Pro Ser Gly Ser Ala Ala Ser Ala Gly Ser 35 40 45

Ala	Ala 50	Gly	Ala	Leu	Lys	Ser 55	Ser	Asn	Asn	Ser	Gly 60	Arg	Ile	Ser	Leu
Leu 65	Leu	Asp	Asp	Val	Asp 70	Asn	Glu	Met	Ala	Ala 75	Ile	Ala	Met	Gln	Gly 80
Phe	Arg	Ser	Met	11e 85	Glu	Gln	Phe	Asn	Val 90	Asn	Asn	Pro	Ala	Thr 95	Ala
Lys	Glu	Leu	Gln 100	Ala	Met	Glu	Ala	Gln 105	Leu	Thr	Ala	Met	Ser 110	Asp	Gln
Leu	Val	Gly 115	Ala	Asp	Gly	Glu	Leu 120	Pro	Ala	Glu	Ile	Gln 125	Ala	Ile	Lys
Asp	Ala 130	Leu	Ala	Gln	Ala	Leu 135	Lys	Gln	Pro	Ser	Ala 140	Asp	Gly	Leu	Ala
Thr 145	Ala	Met	Gly	Gln	Val 150	Ala	Phe	Ala	Ala	Ala 155	Lys	Val	Gly	Gly	Gly 160
Ser	Ala	Gly	Thr	Ala 165	Gly	Thr	Val	Gln	Met 170	Asn	Val	Lys	Gln	Leu 175	Tyr
Lys	Thr	Ala	Phe 180	Ser	Ser	Thr	Ser	Ser 185	Ser	ser	Tyr	Ala	Ala 190	Ala	Leu
Ser	Asp	Gly 195	Tyr	Ser	Ala	Tyr	Lys 200	Thr	Leu	Asn	Ser	Leu 205	Tyr	Ser	Glu
Ser	Arg 210	Ser	Gly	Val	Gln	Ser 215	Ala	Ile	Ser	Gln	Thr 220	Ala	Asn	Pro	Ala
Leu 225	Ser	Arg	Ser	Val	Ser 230	Arg	ser	Gly	Ile	Glu 235	Ser	Gln	Gly	Arg	Ser 240
Ala	Asp	Ala	Ser	Gln 245	Arg	Ala	Ala	Glu	Thr 250	Ile	Val	Arg	Asp	Ser 255	Gln
Thr	Leu	Gly	Asp 260	Val	Tyr	Ser	Arg	Leu 265	Gln	Val	Leu	Asp	Ser 270	Leu	Met
Ser	Thr	Ile 275	Val	Ser	Asn	Pro	Gln 280	Ala	Asn	Gln	Glu	Glu 285	Ile	Met	Gln
Lys	Leu 290	Thr	Ala	ser	Ile	Ser 295	Lys	Ala	Pro	Gln	Phe 300	Gly	Tyr	Pro	Ala
Val 305	Gln	Asn	Ser	Val	Asp 310	Ser	Leu	Gln	Lys	Phe 315	Ala	Ala	Gln	Leu	Glu 320
Arg	Glu	Phe	Val	Asp 325	Gly	Glu	Arg	Ser	Leu 330	Ala	Glu	Ser	Gln	Glu 335	Asn
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Ala Ser Leu Phe Ser Gly Tyr Leu Ser 355

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<211> 207 <212> PRT

<213> Chlamydia

<400> 300

Ser Ser Lys Ile Val Ser Leu Cys Glu Gly Ala Val Ala Asp Ala Arg

Met Cys Lys Ala Glu Leu Ile Lys Lys Glu Ala Asp Ala Tyr Leu Phe

Cys Glu Lys Ser Gly Ile Tyr Leu Thr Lys Lys Glu Gly Ile Leu Ile

Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu

Arg Asn Tyr Phe Arg Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe 115 120

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala 135

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu 145 150

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr 170

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu

Tyr Gly Pro Phe Leu Gln Ala Val Thr Trp Ser Gln Glu Lys Lys 200

<210> 301

<211> 183

<212> PRT <213> Chlamydia

<400> 301

Ile Pro Pro Ala Pro Arg Gly His Pro Gln Ile Glu Val Thr Phe Asp 10

Ile Asp Ala Asn Gly Ile Leu His Val Ser Ala Lys Asp Ala Ala Ser Gly Arg Glu Gln Lys Ile Arg Ile Glu Ala Ser Ser Gly Leu Lys Glu Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu Asp Lys Gln Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile Pro Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala 135 Gln Ser Ala Ser Ala Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly 145 150 155 Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 170 Pro Pro Ala Gly Gly Ser Ala 180 <210> 302 <211> 232 <212> PRT <213> Chlamydia <400> 302 Met Thr Lys His Gly Lys Arg Ile Arg Gly Ile Gln Glu Thr Tyr Asp Leu Ala Lys Ser Tyr Ser Leu Gly Glu Ala Ile Asp Ile Leu Lys Gln

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35 40 45 Asp Pro Arg Lye Ser Asp Gln Gln The Arg Gly Ser Val Ser

Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser 50 55 60

Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala 65 70 75 80

Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly 85 90 95

- Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp
- Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly 115 120 125
- Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr 130 135 140
- Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys 145 \$150\$
- Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala
- Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu 180 185 190
- Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200 205
- Leu Val Asn Phe Thr Ile Ser Ser Thr Met Gly Pro Gly Val Thr Val 210 215 220
- Asp Thr Arg Glu Leu Ile Ala Leu 225 230
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- Ile Lys Lys Ser Phe Lys Met Gly Asn Ser Gly Phe Tyr Leu Tyr Asn 20 25 30
- Thr Gln Asn Cys Val Phe Ala Asp Asn Ile Lys Val Gly Gln Met Thr  $35 \hspace{1cm} 40 \hspace{1cm} 45$
- Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro 50 60
- Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser 65 70 75 80
- Asn Asn Pro Ser Thr Asn Ala Ser Ile Thr Ile Gly Leu Asp Ala Glu 85 90 95
- Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly 100 105 110
- Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp 115 120 125

Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val 165 170 Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile Ala Ser Arg Met Glu Gly Gly Val Val Leu Ala Leu Val Arg Glu Gly 195 200 Asp Ser Lys Pro Tyr Ala Ile Ser Tyr Gly Tyr Ser Ser Gly Val Pro 215 Asn Leu Cys Ser Leu Arg Thr Arg Ile Ile Asn Thr Gly Leu 230 <210> 304 <211> 133 <212> PRT <213> Chlamydia <400> 304 His Met His His His His His Met Ala Ser Ile Cys Gly Arg Leu Gly Ser Gly Thr Gly Asn Ala Leu Lys Ala Phe Phe Thr Gln Pro Ser Asn Lys Met Ala Arg Val Val Asn Lys Thr Lys Gly Met Asp Lys Thr Val Lys Val Ala Lys Ser Ala Ala Glu Leu Thr Ala Asn Ile Leu Glu 50 Gln Ala Gly Gly Ala Gly Ser Ser Ala His Ile Thr Ala Ser Gln Val Ser Lys Gly Leu Gly Asp Thr Arg Thr Val Val Ala Leu Gly Asn Ala Phe Asn Gly Ala Leu Pro Gly Thr Val Gln Ser Ala Gln Ser Phe Phe Ser His Met Lys Ala Ala Ser Gln Lys Thr Gln Glu Gly Asp Glu Gly

130 <210> 305 <211> 125

Leu Thr Ala Asp Leu

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Lys	Ala	Phe	Phe 20	Thr	Gln	Pro	Ser	Asn 25	Lys	Met	Ala	Arg	Val 30	Val	Asn	
Lys	Thr	Lys 35	Gly	Met	Asp	Lys	Thr 40	Val	Lys	Val	Ala	Lys 45	Ser	Ala	Ala	
Glu	Leu 50	Thr	Ala	Asn	Ile	Leu 55	Glu	Gln	Ala	Gly	Gly 60	Ala	Gly	Ser	Ser	
Ala 65	His	Ile	Thr	Ala	Ser 70	Gln	Val	Ser	Lys	Gly 75	Leu	Gly	Asp	Thr	Arg 80	
Thr	Val	Val	Ala	Ļeu 85	Gly	Asn	Ala	Phe	Asn 90	Gly	Ala	Leu	Pro	Gly 95	Thr	
Val	Gln	Ser	Ala 100	Gln	Ser	Phe	Phe	Ser 105	His	Met	Lys	Ala	Ala 110	Ser	Gln	
Lys	Thr	Gln 115	Glu	Gly	Asp	Glu	Gly 120	Leu	Thr	Ala	Asp	Leu 125				
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ccatcaca	ct :	ggcg	gccg	ct c	atgt	ttat	a ac	aaag	gaac	tta	tgaa	tcg	agtt	atagaa	480
atccatgo	etc :	acta	cgat	ca a	agac	aact	t tc	tcaa	tctc	caa	atac	aaa	cttc	ttagta	540
catcatco	tt :	atct	tact	ct t	atto	ccaa	g tt	tcta	ctag	gag	ctct	aat	cqtc	tatget	600
ccttatto	at :	ttac	agaa	at q	gaat	tage	t at	ttet	ggac	ata	aaca	agg	taaa	gatega	660
gatacctt															720
ctcatact															780
ctagcagg															840
attaatat															900
ttacqaaa															960
															1020
gacatete															
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caaattac															1260
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gcttccct	aq a	atcqa	acac	aa t	tcta	tctt	a at	caaa	qaaq	ctc	ccta	taa	aatc	caactt	1740
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Met His		tri a	***	***	wie	m)	21.	21.		2	2	Dha	<b>01</b> -	T	
nec nis	nis	птв	5	nis	nis	THE	MIA	10	ser	Asp	ASII	Pile	15	пеп	
	a1	<b>~1</b>		<b>~1</b>	Dh.a	71.	T1 -		T1 -	a1	<b>01</b> =	77.		71-	
Ser Gln	GIA		GIN	GIY	Pne	Ala		Pro	TIE	GIY	GIN		met	Ala	
		20	~ .				25					30	m)		
Ile Ala		GIn	He	Lys	Leu		Thr	Val	His	Ile		Pro	Thr	Ala	
	35					40	_	_		_	45		_		
Phe Leu	GlÀ	Leu	Gly	Val		Asp	Asn	Asn	GIY		GIA	Ala	Arg	Val	
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Gln Arg	Val	Val	Gly		Ala	Pro	Ala	Ala	Ser	Leu	Gly	Ile	Ser	Thr	
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Gly Asp	Val	Ile	Thr	Ala	Val	Asp	Gly	Ala	Pro	Ile	Asn	Ser	Ala	Thr	
			85					90					95		
Ala Met	Ala	Asp	Ala	Leu	Asn	Gly	His	His	Pro	Gly	Asp	Val	Ile	Ser	
		100				-	105			-	_	110			
Val Thr	Trn	Gln	Thr	Lvs	Ser	Glv	Glv	Thr	Ara	Thr	Glv	Asn	Val	Thr	
	115	,		,		120	1				125				
Leu Ala		Glv	Pro	Pro	Δla		Dhe	Cve	Arc	Tur		Ser	Hie	Trn	
130	- Lu	OT.	210	210	135	JIU	ZIE	-ya	AL 9	140	210	Set	4110	-1P	
Arg Pro	T 011	Mot	Dhe	T1 c		T are	G1	T 011	Mot		70.00	Ma 2	T1c	C1	
	Leu	nec	rne		THE	nys	GIU	ned		ASI	wrd	val	тте		
145	.1.	***	m	150	<b>61</b> .		<b>a</b> 1.		155	a1	0	D		160	

Ile His Ala His Tyr Asp Gln Arg Gln Leu Ser Gln Ser Pro Asn Thr 165 170 175 Asn Phe Leu Val His His Pro Tyr Leu Thr Leu Ile Pro Lys Phe Leu

Leu Gly Ala Leu Ile Val Tyr Ala Pro Tyr Ser Phe Ala Glu Met Glu

185 190

Leu Ala Ile Ser Gly His Lys Gln Gly Lys Asp Arg Asp Thr Phe Thr 215 Met Ile Ser Ser Cys Pro Glu Gly Thr Asn Tyr Ile Ile Asn Arg Lys 230 235 Leu Ile Leu Ser Asp Phe Ser Leu Leu Asn Lys Val Ser Ser Gly Gly 245 250 Ala Phe Arg Asn Leu Ala Gly Lys Ile Ser Phe Leu Gly Lys Asn Ser 265 Ser Ala Ser Ile His Phe Lys His Ile Asn Ile Asn Gly Phe Gly Ala 275 280 285 Gly Val Phe Ser Glu Ser Ser Ile Glu Phe Thr Asp Leu Arg Lys Leu 295 300 Val Ala Phe Gly Ser Glu Ser Thr Gly Gly Ile Phe Thr Ala Lys Glu 310 315 Asp Ile Ser Phe Lys Asn Asn His His Ile Ala Phe Arg Asn Asn Ile 325 330 335 Thr Lys Gly Asn Gly Gly Val Ile Gln Leu Gln Gly Asp Met Lys Gly 345 Ser Val Ser Phe Val Asp Gln Arg Gly Ala Ile Ile Phe Thr Asn Asn 360 Gln Ala Val Thr Ser Ser Ser Met Lys His Ser Gly Arg Gly Gly Ala 375 380 Ile Ser Gly Asp Phe Ala Gly Ser Arg Ile Leu Phe Leu Asn Asn Gln 390 395 Gln Ile Thr Phe Glu Gly Asn Ser Ala Val His Gly Gly Ala Ile Tyr 405 410 Asn Lys Asn Gly Leu Val Glu Phe Leu Gly Asn Ala Gly Pro Leu Ala 420 425 430 Phe Lys Glu Asn Thr Thr Ile Ala Asn Gly Gly Ala Ile Tyr Thr Ser 435 440 445 Asn Phe Lys Ala Asn Gln Gln Thr Ser Pro Ile Leu Phe Ser Gln Asn 455 460 His Ala Asn Lys Lys Gly Gly Ala Ile Tyr Ala Gln Tyr Val Asn Leu 470 475 Glu Gln Asn Gln Asp Thr Ile Arg Phe Glu Lys Asn Thr Ala Lys Glu 485 490 Gly Gly Gly Ala Ile Thr Ser Ser Gln Cys Ser Ile Thr Ala His Asn 500 505 Thr Ile Thr Phe Ser Asp Asn Ala Ala Gly Asp Leu Gly Gly Gly Ala 520 525 Ile Leu Leu Glu Gly Lys Lys Pro Ser Leu Thr Leu Ile Ala His Ser 535 540 Gly Asn Ile Ala Phe Ser Gly Asn Thr Met Leu His Ile Thr Lys Lys 550 555 Ala Ser Leu Asp Arg His Asn Ser Ile Leu Ile Lys Glu Ala Pro Tyr 565 570 Lys Ile Gln Leu Ala Ala Asn Lys Asn His Ser Ile His Phe Phe Asp 585 590 Pro Val Met Ala Leu Ser Ala Ser Ser Ser Pro Ile Gln Ile Asn Ala 600 Pro Glu Tyr Glu Thr Pro Phe Phe Ser Pro Lys 615

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vara cirrum,	yara cracin	JIIICELD				
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cagggattcg c						120
accgttcata t						180
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ggcgacgtga t						300
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ggcacgcgta c						420
ccatcacact g						480
gcatcatctt c						540
aagggtatga t aatagaacat c						600 660
						720
aatggagccc a						720
ccaggatect c						840
caagcagaga t						900
gatcctgaag g						960
atcttgctca c						1020
acgaacaaac a						1020
aacaatacta a						1140
gaatccaatc g						1200
cagacageet e						1260
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ttttccaaga c	caaaacttct	aaacatcgcc	atccccatag	ggattggtta	tgaattctgc	1800
ttagggaata g						1860
aaacgagaaa a						1920
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aaagcatgtt c	cttatacat	cacggcatat	actatcaacc	gtgaagggaa	gaacctctcc	2040
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Pro	Glu	His	Ile	Thr	Ser	Glu	Glv	Ile	Val	Gln	Asn	Val	Glv	Leu	Thr	
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His	Val	Trn	Glv	Pro	Len	Thr	Va 1	Asn		Thr	Len	Cvs	Δla	Ala	Len	
			500					505			200	0,0	510	*****	Lou	
Aen	uie	λen		Met	Val	Ara	Tla		Car	Luc	Ture	Aen		Thr	Tree	
лэр	1113	515	ALG	ricc	Val	Arg	520	Cys	Ser	Був	Був	525	шъ	1111	TYL	
G1	T		7	mla sa	Dha	<b>61</b>			<b>61</b>	m1	*		77-		m	
GLY	530	пр	Asp	1111	FILE	535	Mec	ALG	Gry	1111	540	GIY	ма	Ser	TYL	
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TIE	GIU	ATA	Thr		тте	Leu	GIn	Arg		Pne	Thr	GIU	inr	Gly	Tyr	
_	_	_	_	565	_	_		_	570	_	_			575	_	
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Ile	Gly	Ile	Gly	Tyr	Glu	Phe	Cys	Leu	Gly	Asn	Ser	Ser	Phe	Ala	Leu	
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\Z_13	, C.	ıramı	ula	crac	-11Oilla	CIS										
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Ile Ala Gly	Gln	Ile	Lys	Leu	Pro	Thr	Val	His	Ile	Gly	Pro	Thr	Ala
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Ala Met Ala	Aer		Len	Agr	Glv	Hie		Pro	GIV	Δer	Val		Ser
Ara Het Ara	100	urd	Leu	nau	Ory	105	III S	FIU	OLY	лор	110	rie	Ser
Val Thr Trp		The	Tarc	Car	G1		Thr	7~~	The	G11-		17-7	Thr
115		1111	ьys	Ser	120	GTA	THE	Arg	THE	125	ASII	vall	1111
Leu Ala Glu		Dro	Drc	212		Dhe	Care	Arc	There		Car	Wie	Ten
neu wia Giu	GIY	PLO		Ala		PHE	cys	arg	140	PIO	ser	nis	TTP

170 175

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	Asp	Ile	Arg	Asp		Gly	Pro	Val	Tyr 490		Leu	Asn	Asn	Ser	
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Tyr	Ile		500 Thr	Gly	Thr	Gly		505 Ile	Val	Phe	Asn		510 Asn	Val	Val
Phe	Thr	515 Leu	Asp	Gly	Asn		520 Leu	Gly	Lys	Arg		525 Leu	Phe	His	Ile
Asn	530 Asn	Asn	Glu	Ile	Thr	535 Pro	Tyr	Thr	Leu	ser	540 Leu	Gly	Ala	Lys	Lys
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	Thr			565					570					575	
Glu	Asn	Thr	Ser 580	Asn	Asn	Pro	Pro	Ser 585	Pro	Thr	Ser	Arg	Asn 590	Thr	Ile
Thr	Val	Asn 595	Pro	Glu	Thr	Glu	Phe 600	Ser	Gly	Ala	Val	Val 605	Phe	Ser	Tyr
Asn	Gln 610	Met	Ser	Ser	Asp	Ile 615	Arg	Thr	Leu	Met	Gly 620	Lys	Glu	His	Asn
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315

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<213> Chlamydia trachomatis

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                   410 415
Ser Gly Ser Gln Asn Ser Ile Thr Glu Lys Ile Thr Leu Glu Asn Gly
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                        425 430
Ser Phe Ile Phe Glu Arg Asn Gln Ala Asn Lys Arg Gly Ala Ile Tyr
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Ser Pro Ser Val Ser Ile Lys Gly Asn Asn Ile Thr Phe Asn Gln Asn
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Thr Ser Thr His Asp Gly Ser Ala Ile Tyr Phe Thr Lys Asp Ala Thr
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Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn Asn Val Thr Ala
           485 490
Thr Gln Ala Ser Ser Ala Thr Ser Gly Gln Asn Thr Asn Thr Ala Asn
         500 505
Tyr Gly Ala Ala Ile Phe Gly Asp Pro Gly Thr Thr Gln Ser Ser Gln
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                      520
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Ser Asn Asn Ser Leu Gln Asn Asn Gln Gly Asp Thr Pro Ala Ser Lys
               550
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Fhe Cys Ser Ile Ala Gly Tyr Val Lys Leu Ser Leu Gln Ala Ala Lys
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Gly Lys Thr Ile Ser Phe Phe Asp Cys Val His Thr Ser Thr Lys Lys
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Thr Gly Ser Thr Gln Asn Val Tyr Glu Thr Leu Asp Ile Asn Lys Glu
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Glu Asn Ser Asn Pro Tyr Thr Gly Thr Ile Val Phe Ser Ser Glu Leu
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<213> Chlamydia trachomatis

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40 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val 55 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr 75 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr 85 90 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 105 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 115 120 125 Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp 135 140 Arg Pro Leu Gly Thr Ile Val Phe Ser Ser Glu Leu His Glu Asn Lys 150 155 Ser Tyr Ile Pro Gln Asn Ala Ile Leu His Asn Gly Thr Leu Val Leu 165 170 175 Lys Glu Lys Thr Glu Leu His Val Val Ser Phe Glu Gln Lys Glu Gly 185 180 190 Ser Lys Leu Ile Met Glu Pro Gly Ala Val Leu Ser Asn Gln Asn Ile 195 200 205 Ala Asn Gly Ala Leu Ala Ile Asn Gly Leu Thr Ile Asp Leu Ser Ser 215 220 Met Gly Thr Pro Gln Ala Gly Glu Ile Phe Ser Pro Pro Glu Leu Arg 230 235 Ile Val Ala Thr Thr Ser Ser Ala Ser Gly Gly Ser Gly Val Ser Ser 250 Ser Ile Pro Thr Asn Pro Lys Arg Ile Ser Ala Ala Val Pro Ser Gly 265 Ser Ala Ala Thr Thr Pro Thr Met Ser Glu Asn Lys Val Phe Leu Thr 280 Gly Asp Leu Thr Leu Ile Asp Pro Asn Gly Asn Phe Tyr Gln Asn Pro 295 300 Met Leu Gly Ser Asp Leu Asp Val Pro Leu Ile Lys Leu Pro Thr Asn 310 315 Thr Ser Asp Val Gln Val Tvr Asp Leu Thr Leu Ser Glv Asp Leu Phe 330 Pro Gln Lys Gly Tyr Met Gly Thr Trp Thr Leu Asp Ser Asn Pro Gln 340 345 350 Thr Gly Lys Leu Gln Ala Arg Trp Thr Phe Asp Thr Tyr Arg Arg Trp 355 360 365 Val Tyr Ile Pro Arg Asp Asn His Phe Tyr Ala Asn Ser Ile Leu Gly 375 380 Ser Gln Asn Ser Met Ile Val Val Lys Gln Gly Leu Ile Asn Asn Met 390 395 400 Leu Asn Asn Ala Arg Phe Asp Asp Ile Ala Tyr Asn Asn Phe Trp Val 410 415 4.05 Ser Gly Val Gly Thr Phe Leu Ala Gln Gln Gly Thr Pro Leu Ser Glu 425 Glu Phe Ser Tyr Tyr Ser Arg Gly Thr Ser Val Ala Ile Asp Ala Lys 440 Pro Arg Gln Asp Phe Ile Leu Gly Ala Ala Phe Ser Lys Ile Val Gly 460 455 Lys Thr Lys Ala Ile Lys Lys Met His Asn Tyr Phe His Lys Gly Ser 470 475 Glu Tyr Ser Tyr Gln Ala Ser Val Tyr Gly Gly Lys Phe Leu Tyr Phe 490 Leu Leu Asn Lys Gln His Gly Trp Ala Leu Pro Phe Leu Ile Gln Gly

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Ser	Ile 530	His	Glu	Arg	Asn	Lys 535	Gly	Asp	Trp	Glu	Asp 540	Leu	Gly	Trp	Leu	
Ala	Asp	Leu	Arg	Ile	Ser	Met	Asp	Leu	Lys	Glu	Pro	Ser	Lys	Asp	Ser	
545					550					555					560	
			Ile	565					570					575		
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Cys	Ala	Tyr 595	Arg	Asn	Leu	Ser	Leu 600	Pro	Val	Gly	Cys	Ala 605	Val	Glu	Gly	
Ala	Ile 610	Met	Asn	Cys	Asn	Ile 615	Leu	Met	Tyr	Asn	Lys 620	Leu	Ala	Leu	Ala	
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Trp	Thr	Leu 675	Tyr	Gly	Asn	Tyr	Thr 680	Ile	Asp	Val	Gly	Met 685	Tyr	Thr	Leu	
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<213> Chlamydia trachomatis

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Leu Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe Phe Gly	
Glu Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn Gly Gly	
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325 330 335	
Leu Val Glu Val Asn Ile Ser Val Glu Lys Gly Gly Ser Val Tyr Ala 340 345 350	
Lys Glu Arg Val Ser Leu Glu Asn Val Thr Glu Ala Thr Phe Ser Ser 355 360 365	
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Ala Thr Ala Val Lys Gln Cys Leu Asp Glu Glu Met Ile Val Leu Leu	
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435 440 445	
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<213> Chlamydia trachomatis

225

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Gly Gly Gly Ala Tyr Val Thr Gln Thr Met Ser Val Thr Asn Thr Thr

235

Ser Glu Ser Ile Thr Thr Pro Pro Leu Val Gly Glu Val Ile Phe Ser 245 250 Glu Asn Thr Ala Lys Gly His Gly Gly Gly Ile Cys Thr Asn Lys Leu 260 265 Ser Leu Ser Asn Leu Lys Thr Val Thr Leu Thr Lys Asn Ser Ala Lys 280 285 Glu Ser Gly Gly Ala Ile Phe Thr Asp Leu Ala Ser Ile Pro Thr Thr 295 300 Asp Thr Pro Glu Ser Ser Thr Pro Ser Ser Ser Pro Ala Ser Thr 310 315 Pro Glu Val Val Ala Ser Ala Lys Ile Asn Arg Phe Phe Ala Ser Thr 325 330 Ala Glu Pro Ala Ala Pro Ser Leu Thr Glu Ala Glu Ser Asp Gln Thr 345 Asp Gln Thr Glu Thr Ser Asp Thr Asn Ser Asp Ile Asp Val Ser Ile 360 365 Glu Asn Ile Leu Asn Val Ala Ile Asn Gln Asn Thr Ser Ala Lys Lys 375 380 Gly Gly Ala Ile Tyr Gly Lys Lys Ala Lys Leu Ser Arg Ile Asn Asn 390 395 Leu Glu Leu Ser Gly Asn Ser Ser Gln Asp Val Gly Gly Leu Cys 405 410 Leu Thr Glu Ser Val Glu Phe Asp Ala Ile Gly Ser Leu Leu Ser His 420 425 Tyr Asn Ser Ala Ala Lys Glu Gly Gly Val Ile His Ser Lys Thr Val 440 445 Thr Leu Ser Asn Leu Lys Ser Thr Phe Thr Phe Ala Asp Asn Thr Val 455 460 Lys Ala Ile Val Glu Ser Thr Pro Glu Ala Pro Glu Glu Ile Pro Pro 465 470 475 Val Glu Gly Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn Thr 485 490 Glu Gly Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp Thr 505 510 Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr Ser 520 525 515 Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr Gln 535 540 Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser Asn 550 555 Glu Asn Thr Asp Glu Ser Ser Asp Ser His Thr Glu Glu Ile Thr Asp 565 570 Glu Ser Val Ser Ser Ser Ser 580

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	ttactgc atcttctga				720
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TIE WIM GIA GL	1 Ile Lys Leu Pro	int val fils	ite Giy Pro	INT ALA	

35 40 45 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val

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Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
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Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
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Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
                        120
                                   125
Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp
                   135 140
Arg Pro Leu Asp Gln Ser Asn Glu Asn Thr Asp Glu Ser Ser Asp Ser
              150 155
His Thr Glu Glu Ile Thr Asp Glu Ser Val Ser Ser Ser Ser Lys Ser
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Gly Ser Ser Thr Pro Gln Asp Gly Gly Ala Ala Ser Ser Gly Ala Pro
                              185
Ser Gly Asp Gln Ser Ile Ser Ala Asn Ala Cys Leu Ala Lys Ser Tyr
                         200
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Val Thr Ala Ser Ser Asp Asn Pro Asp Ser Ser Ser Ser Gly Asp Ser
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225
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Glu Thr Pro Thr Leu Ile Gly Gly Gly Ala Ile Tyr Gly Glu Thr Val
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Lys Ile Glu Asn Phe Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala
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                         280
Ile Asp Asn Thr Thr Glu Gly Ser Ser Lys Ser Asn Val Leu Gly
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Gly Ala Val Tyr Ala Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser
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Thr Gly Gln Val Ala Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile
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Asn Ala Thr Asp Thr Gln Arg Lys Asp Thr Phe Gly Gly Ala Ile Gly
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Val Ala Asp Leu Gly Ser Ala Ile Gly Leu Val Pro Asp Thr Gln Asn
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Lys Ala Leu Lys Arg Ala Thr Ile Tyr Ala Pro Val Val Ser Ile Lys
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Ala Ile Tyr Phe Thr Lys Glu Ala Ser Ile Glu Ser Leu Gly Ser Val
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Leu Phe Thr Gly Asn Leu Val Thr Pro Thr Leu Ser Thr Thr Thr Glu
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Ile Phe Gly Gln Ile Ala Ser Ser Asn Gly Ser Gln Thr Asp Asn Leu
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Glu	Gln	Lys	Glu 660	Gly	Ser	Ser	Leu	Val 665	Met	Thr	Pro	Gly	Ser 670	Val	Leu
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